AN EVALUATION OF TETRACYCLINE STAIN REMOVAL BY BLEACHING VITAL RABBIT INCISORS

by

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INTRODUCTION

In 1956 tetracyclines were first implicated in the intrinsic staining of teeth being formed at the time of administration of this family of drugs. Even though only a limited portion of the population is affected, this very unesthetic staining can be damaging psychologically.[1,2,3] Spencer[4] suggested the use of an acrylic veneer, acid-etched to the labial surfaces of these teeth, to mask the stain, but the sole treatment for permanent improvement of this affliction is still the porcelain veneer crown.

Several clinicians have attempted to bleach the tetracycline stain from the teeth. Although monthly re-bleaching was necessary, Cohen and Parkins[5] reported in 1970 that in five of six patients esthetic improvement was achieved by bleaching the tetracycline-stained teeth with concentrated hydrogen peroxide and heat. Arens, Rich and Healey[6] in 1972 reported that three of five patients showed marked esthetic improvement after bleaching their patients' teeth with concentrated hydrogen peroxide and heat. They also stated that the yellow and brown hues of tetracycline staining bleached more successfully than the gray hues.

No reports have been published offering evidence as to why only yellow and yellow-brown teeth, as opposed to teeth stained gray, respond to the bleaching procedure.

The bleaching process is not fully understood: there is a question as to whether its effect is due to the depth of penetration of the bleaching

agent into the affected tooth, to a masking effect of the stain in the tooth, or to some other factor.

It is hypothesized (1) that the procedure used in the clinical bleaching of tetracycline-stained teeth alters the tetracycline in the stained tooth to a less fluorescent state throughout the crown, with no distinct boundaries in the enamel or dentin, and (2) that the strongest bleaching occurs at the dentino-enamel junction.

This study was designed to investigate the effectiveness of the bleaching technique described by Cohen and Parkins[5] and Arens, Rich and Healey[6] by measuring the tetracycline fluorescence in ground sections from rabbit incisors. The fluorescence was measured both in location and in amount by using an ultraviolet light microscope coupled to a specifically designed television electronic measurement system.

REVIEW OF THE LITERATURE

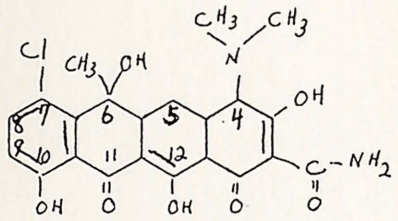
I. The Tetracyclines

Broad spectrum antibiotics came into medical use after Duggar in 1948[7] isolated chlortetracycline from Streptomyces auriofaciens and it became the third major antibiotic to be discovered, after penicillin and streptomycin. A number of analogues of the basic molecule have been derived either from active chemical variants in the fermentation products or from synthetic chemical alterations. The eight major analogues of tetracycline are chlortetracycline (CTC), oxytetracycline (OTC), tetracycline (TC), demethylchlortetracycline (DMTC), N-(pyrrolidinomethyl)-tetracycline (PMTC), methacycline (MTC), doxycycline (DTC), and minocycline (MITC).[8] These differ in the position of chloride, hydroxyl, methyl, or other organic radicals on positions five, six and seven of the basic naphthacene ring system. Also, these radicals represent the primary basis of deriving the generic name of the various analogues.[9,10]

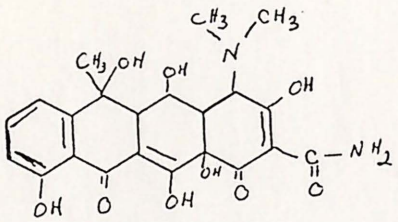
All of these analogues are on the market in the United States. OTC was introduced in 1950 after being prepared from Streptomyces rimosus.[11,12] TC was introduced in 1953 after being prepared by catalytic hydrogenation over palladium of the chlorine radical.[9] DMTC produced by a mutant of Duggar's original strain was described in 1957,[13] and became available in 1959. In 1959 an injectable form PMTC was introduced for those who could not take the drug orally.[14,15,16] MTC and DTC were introduced in 1967.[17,18] MITC came into the market in 1972 after being mentioned in 1966.[18,19] In 1962 6-demethyl-6-deoxytetracycline was synthesized

chemically and was considered a major break-through in pharmaceutical research, although the chemical synthesis is not commercially advantageous.[20]

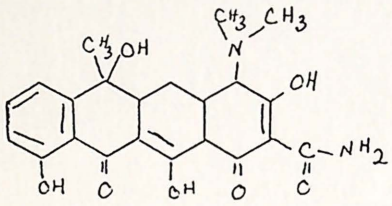
The structural formulas of the tetracyclines are as follows:[15,18, 21,22,23]



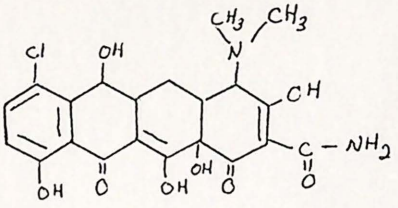
chlortetracycline



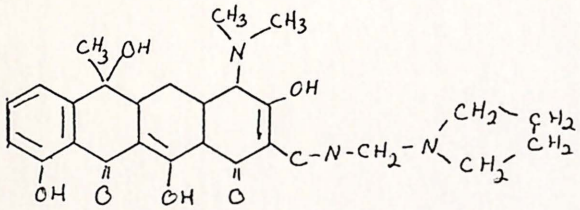
oxytetracycline



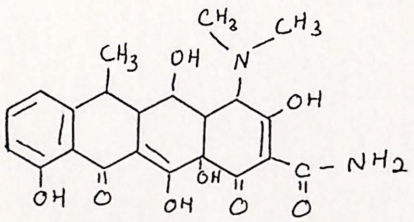
tetracycline



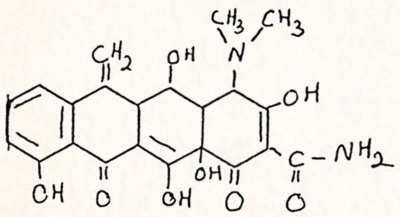
demethylchlortetracycline



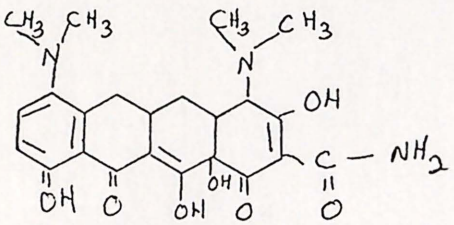
N-(pyrrolidinomethyl)-tetracycline



doxycycline



methacycline



minocycline

The trade names of the various tetracyclines are as follows:[15,16,18,19,21,24,25]

TC-HCl Achromycin, Achromycin V, Kesso-Tetra, Rexamycin, Steclin, Sumycin, Tetrachel, Tetracyn, Panmycin, Polycycline, Cyclomycin, Bristaciclina, Hostacyclin, Omegamycin, Tetradecin, Agromicina, Sanclomycin, Purocyclina, Tetrabon, Criseociclina, Ambramicia, Bristacycline, Cyclopar, Azotrex, Mysteclin-F, Robitet, SK-Tetracycline, Tetracycline, QID-tet, Retet

TC-Phosphate Complex--Tetrex

CTC Aureomycin, Biomycin, Biomitsin, Acromize, Aurecina, Chrysomykeil

OTC Terramycin, Biostate, Ryomycin, Oxy-Kesso-Tetra, Uri-Tet, Azopak, Oxy-Tetrachel, Urobiotic 250

DMTC Declomycin, Demeclocycline

PMTC Tetracycline, Syntetrin, Velacycline, Rolitetracycline, Rerverin

MTC Rondomycin

DTC Vibramycin Hyclate, Vibramycin Monohydrate, Doxy-II

MITC Minocin, Vectrin

The drugs are supplied in oral and parenteral forms except for PMTC, which is used only parenterally.[16] Terramycin is supplied in opaque, yellow, hard gelatin capsules which contain either 250 mg. or 125 mg. OTC-HCl and glucosamine. Terramycin Syrup (calcium oxytetracycline) is available as a preconstituted fruit-flavored aqueous suspension in 5 cc. equalling 125 mg. of OTC and N-acetylglucosamine. Terramycin also is in an intramuscular and an intravenous form;[19,26] however, OTC is the most toxic upon intraperitoneal injection of all the analogues.[27]

A. Physical Properties

As a group, the tetracycline antibiotics are amphoteric crystalline compounds soluble in glycol ethers, pyridine and dilute acid and alkali, very slightly soluble in water and in lower molecular weight alcohols, and insoluble in ether and hydrocarbons. The acid salts (used therapeutically) are well formed crystalline compounds with high solubility in water.[28] The tetracycline analogues will vary as to solubility and stability according to the radicals that are substituted or replaced. CTC is the most unstable of the tetracyclines *in vitro*.[23,29] Of the four major analogues (CTC, OTC, TC, DMTC) DMTC has the greatest resistance to degradation by acids or by alkali.[10,30] PMTC was developed and marketed as more soluble than the original four and thus the best analogue for injection.[16,31]

Terramycin

Terramycin, the analogue which will be used in this study, is a pale yellow, amphoteric substance with a bitter taste. Elemental analysis indicates the emperical formula to be $C_{22}H_{22-24}N_2O_9 \cdot H_2O$. The antibiotic is optically active and gives positive ferric chloride, Pauly, Friedel-Crafts, Fehling and Molisch tests.[11] Its ultraviolet absorption spectrum is maximum at approximately 247, 275 and 353 mμ. It also shows a characteristic absorption in the infrared region. Terramycin and its hydrochloride are moderately soluble in methanol, ethanol, acetone, and propylene glycol. OTC hydrochloride is soluble in water but on standing precipitates to the amphoteric base. OTC is soluble in water to the extent of .25 mg./ml. at 25°C. Aqueous solutions of the hydrochloride

at pH 1.0 to 2.5 are stable for at least thirty days at 5°C and 25°C. Solutions at pH 3.0 to 9.0 show no detectable loss in potency on storage at 5°C for at least one month. In the dry state, OTC and its hydrochloride show no detectable loss in activity on prolonged storage at 25°C; it has less than 5 percent inactivation after four months at 56°C, and no loss in potency on heating for four days at 100°C. OTC has a melting point of approximately 185°C with decomposition $[a]_D^{25}$ --196.6°C (1% in 0.1 N HCl). The pK_a for OTC-HCl are 3.5, 7.6, and 9.2.[11,12,32,33]

The tetracycline structure contains essentially two 1,3 diketones with two of the ketones in the enol form. Such monoenols in 1,3 diketones chelate readily with metallic ions. Drug-metal complexes of 1:1 and 2:1 have been formed with Fe^{+++}, Al^{+++}, Cu^{++}, Ni^{++}, Fe^{++}, Co^{++}, Zn^{++}, Ca^{++}, Mn^{++}. They have also been shown to chelate with organic compounds like sodium salicylate, sodium p-hydrobenzoates, riboflavin, and amino acids.[31,34] The complexes have formed distinctive colors: Cu^{++} and Ni^{++} were green; Fe^{+++} and Fe^{++} were red; and Co^{++}, Zn^{++}, Mn^{++}, and Ca^{++} were yellow.[35] It was shown that the constants for OTC and CTC are almost identical in binding metal cations.[35] It is suggested that such organo-metallic tetracycline complexes are responsible for antimetabolic action of tetracyclines. Magnesium-tetracycline could compete with magnesium for a specific enzymic site in oxidative phosphorylation.[36] Mn^{++} has been found to reverse the inhibitory action of CTC on the nitroreductase activity of E. Coli.[37] It has been shown that the tetracyclines are neutralized by certain metallic cations so that biological activities (which may not necessarily be associated with its chelating activities) are lost.[38]

OTC was found to affect excretion of B vitamins and their activity, and may conceivably involve association with essential metabolites in vivo.[34] The mutual effects of OTC and each of eighteen multivalent inorganic cations, eight multivalent anions, and five antibiotics on the growth of cells of Pseudomonas aeruginosa were studied. The toxicity of OTC was strongly reversed by salts of Fe^{+++}, Fe^{++}, Mg^{++}, Mn^{++}, and $MoO_4^{=}$.[39]

All the tetracyclines fluoresce yellow under ultraviolet light.[40] The amount of fluorescence varies with the analogue used and the administered dose. DMTC and TC fluoresce the most, followed by DTC, MTC, PMTC, OTC, CTC and MITC. This is a qualitative judgement and varies among authors and differs with species and methods of measurement used.[41,42,43]

B. Clinical Pharmacologic Properties

The tetracyclines are rapidly absorbed from the duodenum, ileum and stomach with little absorption from the colon.[18,29,31,32,44-47] Absorption is increased if the analogues are taken in the fasting state.[18] Doxycycline and minocycline are least affected by concurrent administration with food or milk and gastric irritation is diminished if the drug is taken after meals, especially in large doses.[17,18,25] Tetracyclines due to chelation are absorbed poorly in the presence of ions of aluminum hydroxide gels, calcium and calcium-containing foods such as dairy products, iron, magnesium sulfate, and sodium bicarbonate.[18,25,31,48,49] Since the tetracyclines are also implicated in disturbing the absorption of some of the vitamins, the use of B complex concomitantly with the antibiotic is suggested.[50] Excipients such as citric acid or phosphate

compounds significantly increased the blood levels of the tetracyclines; however, these elevated levels were of no real therapeutic value.[28,31,34,51,52] The addition of .5 percent terephatalic acid gave approximately a twofold increase in CTC serum levels.[49]

The greatest differences among analogues is the duration and concentration of blood levels. Half-life in serum for MITC is 11-17 hours; for DTC, 18-22 hours; MTC, 14 hours; DMTC, 12 hours; PMTC, 12 hours; OTC, 9.6 hours; TC, 8.5 hours; and CTC, 5.6 hours.[13,18,19,53] The method of administration has a great deal to do with the amount of tetracycline present, as 6 mg./kg. of OTC given intramuscularly was equal in blood levels to 22 or 33 mg./kg. taken orally, and 25 mg./kg. of OTC intramuscularly was equivalent to 6.6 mg./kg. intravenously.[54,55] Although there are minor differences in the absorption of the different analogues from the gastrointestinal tract therapeutic levels can be maintained by six-hourly administration, the usual dose being 250 mg.[10,13,23,29,32,44,56,57,58]

The administration of tetracycline in pregnancy creates another problem in that the fetus receives only about one-fourth of the maternal dose but due to the relative size of the fetus the dose can be very high, approaching 400 mg./kg.[59,60,61] Tetracyclines given to infants, both premature and full term, in doses of 6 and 12 mg./kg. gave satisfactory therapeutic serum levels.[55,61] Intravenous dosage is 0.5 to 1.0 g./day for an adult and 10 to 20 mg./kg. total daily for a child.[32]

The tetracyclines diffuse well into most body fluids and tissues.[32,62-67] Their distribution in soft tissues is widespread with highest concentrations occurring in the reticuloendothelial system, liver and

kidney.[31,32,46,62-65,67] The analogues appear in the milk of lactating patients.[28,31,43] Tetracyclines cross the placental barrier.[21,26,28,31,32,59,61,68-86] The drug is not seen in fat,[62,63] and has poor penetration of the central nervous system.[31,32,54,63-65,87] Both *in vitro* and *in vivo*, dead and normally mineralized bone does not take up tetracycline.[88] The drug is bound to plasma proteins and this affects the mode of excretion and serum half-life.[18,28,31,89] The distribution of antibiotics in the organs of the human body is the result of several conflicting relations: namely, resorption and excretion on the one hand; and on the other distribution and rate of flow of the circulating blood and the conditions of transfer into the tissue liquor, including penetration of and accumulation in the tissue cells.[90] The tetracyclines are concentrated in the liver, kidney, and in calcifying tissues.[31,46,63,65,91,92]

The principal mechanism of excretion is passive glomerular filtration. TC is concentrated in the urine to the greatest extent, and MITC is the least concentrated. It is evident that the analogue with the lowest excretion rate has the highest blood levels and half-life. In oliguria and renal failure the half-life can be significantly prolonged, i.e. from 8 hours to 108 hours. CTC is not affected significantly by renal failure. The analogues appear in the urine in an active state. The drug is also found in the feces and this is due to incomplete absorption from the intestines as well as the concentration of the drug in the liver and excretion by way of bile. Thus, the tetracycline describes a circuit in the organism: intestine to blood to liver to bile to intestine. There is then about ninety percent excretion by the urinary and fecal

route with another ten percent deposited in bone and calcifying tissue.[17, 18,26,28,29,31,32,44-46,53,56,62,67,92-95]

C. Clinical Antimicrobial Spectrum

The mode of action or the precise mechanism by which the tetracyclines exert their antimicrobial action is unknown. The following effects appear to have been established. First, tetracyclines are active chelating compounds with divalent and trivalent cations, and may thereby interfere with enzymes requiring such cations as cofactors. Next, there is an inhibition of protein synthesis which occurs simultaneously with an increase in the rate of nucleic acid formation within the cell. Finally, the drugs appear to interfere with the phosphorylation of glucose in both bacterial and mammalian cells. The greater sensitivity of bacteria relative to higher animals toward the tetracyclines remains unexplained, except that tetracyclines interfere with both oxidative process and protein synthesis in bacteria, and in man only the oxidative systems seem to be affected.[28,32,36,37,38,96-100]

The tetracyclines are considered to be bacteriostatic but at times appear to exert a bactericidal effect when massive intravenous doses are given.[26-29] Mirror images of the analogues have no antimicrobial activity.[20]

In general, no important differences have been documented in clinical effectiveness of the analogues.[9,18,27,28,31,44,56,57,101-104] The differences are quantitative rather than qualitative. A given organism will be resistant to all analogues if it is resistent to one.[18,56] As is true of many antimicrobial agents, the tetracyclines are frequently administered unnecessarily, such as for viral infections, or are

administered when less toxic or more efficacious antimicrobial agents are available.[2]

The problem of antimicrobial resistance is two fold; on the one hand there is the development of resistant strains during the course of therapy by means of mutation and selection, and on the other there is the superimposition of strains of established resistance after suppression of the susceptible microflora. The tetracyclines have been used for chronic urinary infections but antimicrobial resistance precludes this use now. The drug has been used very successfully to treat on a long-term basis cystic fibrosis,[79,104-108] chronic bronchitis, bronchiectasis and in low dosage for acne.[18,32,109]

Plaza-Roca[110] suggested that bone could act as a depot for storing OTC and several authors have proposed that the bone-seeking properties of the drug could be used to combat osteomyelitis.[111,112] However, Anderson, Ferguson and Braude[113] showed that bone is no longer bacteriostatic 96 hours after the administration of tetracycline and that the persistence of tetracycline fluorescence bears an independent relationship to its pharmacologic effect. Cullen and Hargadon[114] made the point that selection of a drug to which the organism was sensitive was more important than the bone-seeking properties of the tetracyclines. Frost in several articles gave strong evidence that tetracyclines had no advantage over the other antibiotics in treating osteomyelitis due to the architecture of bone.[88,115]

Ory[18] in his review of tetracyclines summarized the use of tetracyclines by the following authors[7,23,26,28,29,32,54,70,94,95,109,116] and is reproduced as a table on the following page.

TABLE I
The clinical antimicrobial spectrum of tetracyclines

Treatment of first choice	Not treatment of first choice but usually effective	Treatment ineffective or more effective therapy available
Diplococcus pneumoniae (if patient is allergic to penicillin)	Diplococcus pneumonia	Endocarditis
Pasteurella tularensis	Streptococcus hemolyticus, Group A (sensitive strains)	Staphylococcal infections, especially bacteremia
Brucellosis	Streptococcus, Group B (sensitive strains)	Gram-negative bacillary bacteremias
Pseudomonas pseudomallei (meliodosis)	Anaerobic streptococci (most strains)	Meningitis
Vibrio cholera (cholera)	Listeria monocytogenes	Chronic osteomyelitis
Bacterioides	Bacillus anthracis (anthrax)	Empyema and suppurative pericarditis
Mycoplasma pneumoniae	Erysiplothrix insidiosa	Septic arthritis
Rickettsial infections	E. coli (some strains)	Tuberculosis
Rocky mountain spotted fever	Hemophilus influenza	Leptospirosis
Typhus fever, murine	Neisseria gonorrhoeae	
Epidemic typhus	Shigella	
Rickettsial pox	Hemophilus Ducrey--chancroid	
Q fever	Pasteurella pestis (plague) combined with streptomycin	
Relapsing fever (Borrelia novyi and Borrelia recurrentis)	Malleomyces mallei (Glanders)	
Psittacosis	Mima-Herellea (some strains)	
Lymphogranuloma venerum granuloma inguinale	Clostridia tetani	
Inclusion conjunctivitis	Clostridia welchii	
Actinomycosis	Treponema pallidum (syphilis)	

Reproduced from Ory[18]

D. Side Effects and Toxicity

Acute toxicity of the tetracyclines is relatively low; the LD_{50} values for intravenous dosage were 170 mg./kg. in mice and 220 mg./kg. in rats for TC.[45] The oral LD_{50} was found to be greater than 3000 mg./kg. in both species. The LD_{50} intravenous dose of OTC in mice was 192 mg./kg.[12,32]

Original therapeutic administrations of the analogues in humans seemed to confirm the consensus that the tetracyclines were relatively innocuous. Several early papers stated that the only side reaction

were gastrointestinal upsets or superimposed infections.[29,32,55,61,70,102,116,117] It is now documented that tetracyclines are not as innocuous as once believed and a review of the major side effects will now be summarized.

Allergic Reactions

Hypersensitivity reactions due to tetracycline and its analogues are rare. A few cases of skin rash or other dermatologic manifestation of reaction to the drugs have been noted.[8,18,19,26,44,81,118,119] There have been typical anaphylactoid reactions to OTC, DMTC, TC, and CTC.[8,120,121]

Phototoxicity

The low allergenicity of the tetracyclines is contrasted by the high incidence of phototoxicity. The reaction manifests itself as an exaggerated sunburn with high fever, eosinophilia, and increased blood platelets, and occurs only on skin exposed to rays in the 2,700 to 3,200 angstrom range. DMTC is usually incriminated but the effect has been reported with other analogues.[8,18,23,26,31,81,105,106,122,123]

Blood

Blood dyscrasias due to tetracycline are rare. The clinical significance of the delay which certain tetracyclines may produce in blood coagulation has not been evaluated fully. There have been reports of anemia, neutropenia, and eosinophilia—but again these are rare and not fully substantiated.[8,18,26,124,125]

Gastrointestinal Tract

Perhaps the most commonly reported side effects are disturbances of the gastrointestinal tract. Nausea, vomiting, anorexia, and epi-

gastric burning occur on oral administration but not on intravenous administration. Stomatitis, cheilosis, vaginitis, proctitis, diarrhea and other enteric symptoms such as flatulence and bulky, loose stools may result from altered bacterial and fungal flora. There have also been oropharyngeal complaints of black hairy tongue, hoarseness, sore throat and a variety of eruptions in and around the mouth from the altered flora. DMTC and MTC probably irritate the gastric mucosa the most, and TC and DTC the least.[8,18,23,28,31,32,44,56,101,102,105,106,116,117]

Hepatic System

Overdosage of intravenously administered tetracycline causes severe liver damage and in some cases death has been reported. A dose of up to two grams per day given intravenously may cause fatal damage of the liver, even in the case of normal kidney function. Pregnant women, children, and those suffering from uropathies are most susceptible.[8,18,28,36,67,76,126,127]

Urinary System

DMTC may damage the kidneys of healthy adults and several other analogues have caused progressive decrease of renal function in patients suffering from renal insufficiency. A progressive decrease in maximum urinary concentration ability and an increase in daily urine volume was noted, as in nephrogenic diabetes, blood urea nitrogen rose and there was a decrease in creatinine clearance. These phenomena regressed upon withdrawal of the drug. Even moderate oral or parenteral doses of tetracyclines given to patients with renal insufficiency have caused further

deterioration of their renal function.[8,18,26,36,128-130] Another problem is the use of degraded tetracycline. It has been documented that outdated tetracyclines cause an adult "Fanconi Syndrome"--renal tubular dysfunction leading to coma.[8,18,131,132] Incriminated is the degradation product epi-anhydrotetracycline.

Central Nervous System

In infants even a single dose of tetracycline may cause acute benign intracranial hypertension (bulging fontanelle). This symptom has also occurred in adults. These are all reversible when the drug is withdrawn.[8, 18,26,133]

Teratogenic Changes

A possible relationship has been reported between high doses of tetracyclines in the first trimester and congenital cataracts in three cases.[134] In another case a women received tetracycline at 33 days gestation and her child was born with deformed hands bilaterally.[135] Tetracyclines also have inhibited growth and disturbed calcification in infants. The inhibition is reversible upon cessation of the drug.[8,36,76,82,136-147] Tetracyclines also deposit in calcifying tissues and this produces an intrinsic stain in teeth if they are forming at the time of the administration. This subject will be discussed in greater detail in a following section.

Miscellaneous Side Effects

Superimposed infections, usually monilia, gram negative infections, and resistant strains of staphylococcal infections, have contributed to fatalities in debilitated patients.[8,18,117] Metabolism of some patients has been disturbed by a catabolic effect and azotemia.[8,18]

II. The Effect of Tetracyclines on Teeth

Regna et al in 1951[33] reported that Terramycin complexed with calcium, but no real significance was attached to this until Andre's[63] excellent study demonstrated that tetracycline was deposited in the skeletons of rats. During the same year, 1956, Schwachman and Schuster[106] published the first indication that the tetracyclines discolored teeth. As time passed more clinicians and researchers began to observe and report cases of tetracycline staining of both primary and permanent teeth, and a few reported hypoplasia of enamel which they attributed to the drug. During During the early 1960's heated debate raged in Lancet's Letter to the Editor column on the disputed subject of tetracycline staining and hypoplasia.[13,85,148-153]

A. Prevalence in the General Population

The prevalence of tetracycline staining in a population varies from country to country and with the sample taken. Witkop, Wolf and Mehaffey[154] found discoloration and fluorescence caused by tetracycline in three separate populations to be 21.2 percent of the children in a pediatric practice, 4.1 percent of urban children and only 1.4 percent of rural children. In children with cystic fibrosis the characteristic staining is from 36 to 83 percent.[78,105-108,155-157] Baker and Storey[158] in 1970 reported that 71 percent of the teeth in children 6 to 7 years of age showed tetracycline deposits. They also noted that the analogues were administered with increasing frequency during 1960 to 1965, with the

peak year being 1962 followed by a decline in usage. A study by Brown[159] confirmed the frequency of use of these drugs. Brearly and Storey[160] compared the results of clinical examinations in visible light with the results of using ultraviolet light to detect macroscopic tetracycline fluorescence and showed that the former provided a better diagnostic criterion for tetracycline staining. They confirmed this by microscopic examination of sample teeth under ultraviolet light which showed that there was microscopic fluorescence in the teeth with no macroscopic fluorescence. Witkop in 1958[161] discussed the results of a survey of 96,471 children in which the different genetic problems of teeth were studied. He found that 15 percent of the children had some variation in color, usually a yellow which he attributed to a mutant strain of enamel. One must also distinguish tetracycline stains from the gray-brown color of amelogenesis imperfecta, the opalescent discoloration in dentinogenesis imperfecta, the bluish-green stain of erythroblastosis fetalis, and the purplish-brown color of congenital porphyria. Extrinisic stains of black, green and orange can also affect the examiner's judgement.[161]

A prevalence study is therefore influenced by a number of external factors. The percentages given for tetracycline discoloration in major studies in the United states are 1.5 percent,[162] 2.3 percent,[163] and 3.5 percent;[164] in Belfast, Ireland, Stewart in 1968[165] reported 7 percent for permanent teeth and 15 percent for primary teeth; and in Australia and nearby regions the percentages of the different studies were 3.4 percent,[166] 17.8 percent,[159] 20.1 percent,[75] and 30 percent.[160]

B. Intrinsic Tetracycline Stain

Authors agree that tetracyclines are deposited along growth planes during odontogenesis, and unlike skeletal tissue, once the drug is fixed it is a permanent stain. Upon completion of calcification and development dental tissues are protected against further staining.[22,83,84,144,146,158,163, 167-174]

Since the tetracyclines cross the placental barrier, the period during which esthetically important tooth discoloration can occur in man ranges from the fourth month *in utero* to approximately the seventh year of life. More specifically, the primary dentition is affected from four months *in utero* to nine months *post-partum* and the permanent dentition after ten months of age to the fifth through the seventh year.[8,18,72,81,84,85,156,163,171,175,176]

In published reports a critical assessment of the severity and the color caused by tetracycline varies between authors due to: a) the analogue used; b) the dosage level of the drug; c) the stage of development and the dentition (primary or permanent) under survey; d) degradation and bleaching of the drugs; e) the length of time and the manner in which the drug was administered; f) the species under survey; g) the presence and severity of any disease from which the drug was given; h) the length of time the affected teeth were exposed to daylight; i) whether the teeth were studied *in vivo* or *in vitro*; and j) examiner variation and subjectivity.

1. Color

Much has been written about which tetracyclines cause the hues of yellow, brown, gray or combinations of these colors. Wallman and Hilton[148]

in 1962 reported that the color varied, young children having yellow pigmentation and older children having brownish colors. OTC was least likely to stain and when it did, it was yellow; TC stained yellow but later turned brown. Weyman[177] in 1963 stated that CTC caused a gray-brown stain and TC, DMTC, and OTC caused a yellow stain which tended to darken to brown. In two other reports, Weyman and Porteous[144,178] encountered two types of staining: one stained with CTC was gray-brown and did not fluoresce in ultraviolet light, and the other stained with TC and OTC was yellow and did fluoresce. They concluded that a yellow tooth erupted yellow, and that a gray-brown tooth erupted slightly darker than normal, but never would appear yellow. Douglas,[74] Storey,[179] Madison,[59] Stewart,[72] and Maculay and Leistyna[77] all stated that the pigmentation was yellow and then turned to a yellowish-brown or brown. Several other authors reported cases in which the teeth were bluish-brown to black.[1,81,157] McIntosh and Storey[180] showed that epianhydrotetracycline (EAHTC), a breakdown product of tetracycline, stained all teeth a very dark brown and that this substance was found in outdated tetracyclines or when tetracyclines were kept in moist and light conditions. Their results show unequivocally that different tetracyclines induce different degrees of discoloration in intact teeth.

Harcourt, Johnson and Storey[181] stated that the teeth are stained by the tetracyclines themselves as the color is identical with the original solution of the drug, it fluoresces at the correct wave length, and it has antibacterial action when released. Owen[82] reported that in dogs the teeth stained with DMTC were very yellow, those with TC and CTC were less yellow, and those with OTC were white or dull white. Thanik and McMurchy

in 1966[141] reported that in dogs DMTC produced a yellow-orange color, TC and CTC produced a yellow color, and OTC gave the least discoloration, a creamy yellow.

2. Effects of Sunlight on Tetracycline Staining

Wallman and Hilton[148] showed that a TC stained tooth which was yellow turned brown on exposure to sunlight. They did this by splitting a TC stained tooth longitudinally and placing one part in sunlight and the other in the dark. They hypothesized that the brown pigmentation was due to an oxidation product of TC. Hilton[111] did the same with TC stained bone. Again the part exposed to sunlight turned brown while the part kept in darkness remained yellow. McIntosh and Storey[180] found that on exposure to light severely affected teeth become gray-brown, those less affected become gray-yellow, and light yellow teeth become gray-white and difficult to distinguish from normal. Brearley and Storey[160] reported that sunlight changed wide yellow bands to dark brown and light yellow bands to gray on sectioned tetracycline stained teeth.

Ibsen, Urist and Sognnaes[24] exposed rabbit teeth stained with different analogues to sunlight. After exposure all teeth turned brown and then CTC, PMTC and OTC upon further exposure returned to normal color. DMTC and TC were more severely stained and remained brown.

Bridges, Owen and Stewart[182] showed that on exposure to daylight all teeth in their study began to discolor further but some more rapidly and to a greater extent than others. Coupled with this degradation was a gradual loss of fluorescent capacity of all samples. Once the color of each tooth had degraded to an observed maximum, it began to bleach. In any single tooth this bleaching occurred more slowly than the degradation.

There was also a differential rate of bleaching. In general, samples which were most stained before exposure to daylight discolored more rapidly and to a greater extent than those whose initial staining was minimal. OTC showed the least initial discoloration, fluorescence and degradation. Tetracycline-L-methylene-lysine (LC) and clomocycline (CC) had the most, and TC and CTC were in between.

Brearley, Stragis, and Storey[75] in another study examined 23 human dentitions which showed a difference in fluorescence and discoloration between anterior and posterior teeth, the posterior teeth being darker than the anterior teeth. They said that alterations of color occurring upon exposure to sunlight appeared to be inadequate to explain their clinical observations.

3. Severity of Staining

McIntosh and Storey[180] grouped the tetracyclines into two groups and in decreasing order of staining. The first group caused severe staining: epianhydrotetracycline (EAHTC), demethychlortetracycline (DMTC), tetracycline (TC), and tetracycline-L-methylene-lysine (LC). The second group caused less staining: chlortetracycline (CTC), methacycline (MTC), doxycycline (DTC), oxytetracycline (OTC), and anhydrotetracycline (AHTC). Three studies on the relative severity of staining in man are in close agreement. Wallman and Hilton[148] stated that TC stains more than OTC; Weyman[177] in 1965 reported a decreasing order of staining in CTC, TC, DMTC, and OTC; and Swallow, DeHaller and Young[155] said that CTC stained more than OTC. In a study of dogs Owens[82] rated in decreasing order of staining DMTC TC, CTC, and OTC; another study in dogs by Thanik and McMurchy[141] arrived at the same order, although the colors were more yellow. Ibsen, Urist

and Sognnaes[24] demonstrated in rabbits in decreasing order of severity DMTC, TC, PMTC, OTC and CTC. Two good studies have been performed in rats. Bridges, Owen, and Stewart[182] found that LC, clomocycline (CC), CTC, TC, and OTC stained in decreasing intensity. The other study by McIntosh and Storey[180] was mentioned earlier.

Johnson in 1964[183] showed that extremely high doses of tetracycline produced brown rather than yellow staining. Evidence has been presented that the total dosage of tetracycline is more important in the discoloration of teeth than the total period of administration.[84,184] The color intensities in bone were directly dose-related.[141] Bevelander and Nakahara[184] stated that in the rat the degree and extent to which dentin and enamel exhibit discoloration as a result of exposure to tetracycline are dependent upon age and dose. Brearley and Storey[160] showed that in human dentitions the quantity of tetracycline administered and the closeness of the staining to the dentino-enamel junction determined the severity of discoloration. A single course of tetracycline given during the formation of the dentino-enamel junction could cause severe discoloration. They also showed that the color of the staining could be altered by the distance the light must pass through enamel and dentin to reach the stained area. This property of light produced different shades and different intensities of color with the same analogue.

Moffitt et al[171] stated that the intensity and severity of the discoloration are influenced by the dosage, duration, and the time of initiation of tetracycline series of antibiotics relative to the period of odontogenesis. The closer to the dentino-enamel junction, the more intense the staining. Bridges, Owens, and Storey[182] showed further that

with higher dosage levels darker discolorations occur. Harcourt, Johnson and Storey[181] showed that coloration was correlated to dose and frequency of administration. Moffitt[156] demonstrated in his thesis that the critical period for tetracycline-related discoloration in the primary dentition was the period of mineralization of the first millimeter of dentin nearest the dentino-enamel junction, which was the first 24 months of life. He also showed that the severity of tooth discoloration was dependent upon duration and total dosage of tetracycline therapy and that the staining became more intense as the period of tetracycline administration began earlier in the child's life. Grossman et al[167] reported that the darkening effect of one course of TC or DMTC during the years of permanent incisor formation was negligible; however, with increasing frequency of tetracycline exposure the risk increased and four of their six patients with eight or more courses had noticeably dark teeth.

Swallow, DeHaller, and Young[155] disagreed stating that there was no statistical difference in discoloration among the analogues OTC, TC, CTC, and DMTC. They further stated that total dosage and time of tooth formation made no statistical difference in the degree of tooth discoloration.

In two cases the dentin was entirely removed from the enamel and discoloration remained in the enamel, particularly in the cervical region.[169,185] Bennett and Law[186] and Bennett[187] showed that tetracycline was incorporated into the calcifying dentin and enamel of dog teeth in a ratio of approximately nine times as much tetracycline in dentin as in enamel. Owen[82] stated that he observed incorporation of tetracycline both in the dentin and enamel at the time of formation. Urist and Ibsen[188]

demonstrated that tetracycline was present in both enamel and dentin, with dentin having the higher incorporation of the drug. Moffitt[156] stated that tetracycline incorporation in enamel was minimal or non-existent in all the primary teeth of his test population, yet tetracycline incorporation was always observed in dentin. Harcourt, Johnson and Storey[181,189] said that they could not demonstrate tetracycline's presence in enamel by fluorescence. Weyman and Porteous[83] were unable to find fluorescence of tetracycline in enamel in human teeth even though it was present in the dentin. Weyman[185] stated in a later study that the stain was found to be in the enamel and was not stain showing through from the dentin. This indicated a higher concentration of tetracycline in the enamel than would be indicated by the amount of fluorescence.

Summary

Intrinsic tetracycline stain is deposited in both enamel and dentin and imparts a yellow hue to the bone or tooth substance. The severity of discoloration is dependent on total dosage, the time of administration in relation to tooth development, the analogue given, and the position of the drug in relation to the dentino-enamel junction. The yellow stain turns brown on exposure to sunlight and the gray hues of tetracycline staining seem to be due to the masking of the stain with varying thicknesses of normal enamel and dentin.

C. Fluorescence: Normal Versus Tetracycline Teeth

Fluorescence is the luminescence which ceases within a very short time (10^{-8} seconds) after the exciting radiation is removed.[190] When a material fluoresces on its own, it is described as having primary fluorescence

or autofluorescence. A material which fluoresces after being impregnated with a fluorescent dye is referred to as having secondary fluorescence.[191] The particular radiation that excites fluorescence and the specific position of that fluorescence in the visible spectrum can be used to identify a substance. This is one of the important physical properties of tetracycline, and a major means of studing the distribution of this drug.

Fluorescent microscopy is accomplished by illuminating the subject under study with ultraviolet light and observing the resulting fluorescence. The ultraviolet light for the microscope is usually generated by filtering out all but the ultraviolet light from the full spectrum of white light.[190-192]

Benedict[193] in 1928 made the following observations in human beings concerning fluorescence. He showed that the lens of the eye was the strongest fluorescing organ, although the teeth were almost as brilliant. He noted that dentin fluoresces more brilliantly than enamel and with a bluer light. Initial dental caries did not fluoresce and the same was true if a tooth was treated with dilute acetic acid. Benedict also found that ashed enamel did not fluoresce and that the organic matrix of dentin retained appreciable fluorescence.

Hals[191] in 1953 wrote an excellent review of the fluorescence of teeth. In fully developed hard tooth tissues the primary fluorescence is strongest in the least mineralized parts. Regions with especially high mineralization, such as the inner zone of the enamel, do not fluoresce. In teeth the cementum fluoresces more than dentin, which fluoresces

more than enamel. Carious regions do not fluoresce, and vital teeth have greater fluorescence than non-vital teeth. The fluorescence of many tissue elements is strongly labile when exposed to ultraviolet light and this is true of both secondary and primary fluorescence. Hals stated that investigations should be done on freshly prepared specimens only in view of the decrease of primary fluorescence in tissues which occurs only a few minutes after death.

From his own research, Hals also reported that the primary fluorescence was blue for enamel and a brighter or whiter blue for dentin. In addition, he referred to a report by Pflüger[194] in 1931 of experimentally induced porphyria in teeth. After repeated injections of uroporphyria in the abdominal skin, the experimental animals were sacrificed and ground sections of the teeth were made and studied with a fluorescence microscope. In longtitudinal sections of the dentin luminous red lines were seen, and in cross-sections rings appeared whose relative position agreed with the time intervals between the injections. Red fluorescence could be observed in the enamel also, but unlike the fluorescence of the dentin there was no striped deposition, but rather a delicate, diffused red tinge in the enamel structure, strongest at the dentino-enamel junction and dimininishing toward the enamel surface where it was not observable. In ordinary light no coloring was demonstrable.

The literature is replete with statements that tetracyclines will cause yellow fluorescence in tissues in which they are deposited.[2,21,22, 24,63-67,74,81,82,110,154,160,163,167,171,174,175,181,189,195-205] Brearley, Stragis and Storey[75] demonstrated that clinical discoloration caused by tetracyclines fluoresced macroscopically in only 84.8 percent of the

cases they studied. Kutscher et al[200] found that patients exhibited fluorescence of greatest intensity when viewed with long-wave ultraviolet light at 366 nanometers and that short-wave ultraviolet light under 253.7 nanometers never gave as good a result. Antalovska and Beran[173] stated that the localization of the fluorescence is not influenced by the type of tetracycline, the dose, or method of administration. Antalovska[170] in another article pointed out that the intensity of macro-fluorescence in dental tissues and in bone varies according to the amount of tetracycline retained. He felt that the amount of tetracycline incorporated could not be determined quantitatively on the basis of the yellow tetracycline fluorescence.

Storey[179] said the drug was incorporated into calcifying dentin and into immature enamel, but appears to be removed or masked as the enamel calcifies, in contrast to its permanent retention in dentin. Harcourt, Johnson, and Storey[181] showed in five children that the tetracycline stain localized in dentin. It fluoresced under ultraviolet light and this fluorescence was associated with a typical globular pattern of calcification in dentin and cementum. Enamel did not fluoresce yellow when viewed directly under the ultraviolet light microscope. Moffitt et al[171] stated that tetracycline fluorescence in enamel was minimal or non-existent. Hammarström[198] reported that the distribution of Ca^{45} in the enamel remained unchanged during the four days of his investigation. The distribution of tetracycline fluorescence, however, was markedly changed with time. One day after injection there was considerable increase in fluorescence in the whole thickness of the enamel. It then gradually decreased and extinction seemed to start at

tips of the cusps and proceeded cervically. After four days, fluorescence could be seen mainly in the cervical parts of the enamel.

Löfgren, Omnell, and Nylen[199] observed that some hypoplastic defects exhibited tetracycline fluorescence while others were negative, as were the incremental bands and the normal enamel. The labelled lesions included all of those caused by the previous injections of tetracycline and not the injection which corresponded to the time of the defect. These authors suggested that the fluorescent lesions labelled by the injection of tetracycline were labelled subsequent to the injection that caused the hypoplastic enamel. Nylen, Omnell, and Löfgren[206] reported that only hypoplastic enamel exhibited the yellow fluorescence of tetracycline.

Harcourt[196] reported on several cases of neonatal jaundice who had received large doses of tetracyclines during the first month of life. Tetracycline fluorescence could be seen in the enamel as well as the dentin of the primary teeth. Both Hefferren et al[205] and Moffitt et al[171] reported that the fluorescence of tetracycline in teeth could be seen most vividly at the cervicals of the teeth due to the intense fluorescence of the dentin which shows through the thinner enamel. They also observed that gray tetracycline-stained teeth had minimal fluorescence, if any, and that brown tetracycline-stained teeth showed less fluorescence than yellow tetracycline-stained teeth.

Hilton[111] exposed TC stained bone to sunlight and after exposure the color turned from yellow to brown and no longer fluoresced under ultraviolet light. Frost[207] has shown that TC labelled bone can be detected and measured after 109 months of life has intervened between labelling and measurement. He obtained his specimen from a patient who had received

TC nine years earlier in life and the bone was removed during surgery. Johnson and Mitchell[43] stated that tetracycline fluorescence fades rapidly after preparation of sections. Mcleay and Walske[112] demonstrated that if the bone which contains the tetracycline is kept in the dark at minus ten degrees centigrade the specimen will maintain its fluorescence and bacteriostatic activity up to six months. Plaza-Roca[110] was able to keep samples for a year and still have fluorescence by storing them in ethyl alcohol in hermetically sealed containers and refrigerated a temperatures normally used for storing food. Fluorescence ceased in specimens exposed for long periods to light and specimens lost fluorescence if exposed to ultraviolet light continuously for thirty minutes to one hour.

A few studies compared the analogues for degree of producing tetracycline fluorescence in teeth. In general, the more severe the discoloration caused by the tetracycline, the more intense the fluorescence. Owen[82] in his study with dogs showed that the decreasing order of fluorescence.was DMTC, TC, CTC, and OTC. Ibsen, Urist, and Sognnaes[24] stated that DMTC, TC, and PMTC were equal in fluorescence and all were more fluorescent than OTC, which was more fluorescent than CTC. Bridges, Owens and Stewart[182] rated the analogues in decreasing order of fluorescence as follows: LC equaled CC, which fluoresced more than TC, which equaled CTC, and the least fluorescing analogue was OTC. Johnson[21] showed in rats that fluorescence decreased from DMTC and CTC to TC and then OTC.

Hodson,[208] upon examination of ground sections of carious tetracycline-banded teeth under light and fluorescent microscopes, found that the yellow-brown pigmented bands and their fluorescence were eliminated by the carious process. Sections treated with dilute lactic acid and

ethylene diamine tetra-acetic acid (EDTA) showed first the quenching of the fluorescent bands, followed later by solution of the drug. Brearly and Storey[160] and Harcourt[196] both reported that dental caries removed the fluorescence in tetracycline-stained dentin.

Summary

Tetracyclines fluoresce yellow when deposited in growing tissue and subjected to ultraviolet light. Dentin fluoresces more than enamel which does not retain the fluorescence of tetracycline. Sunlight and chemical decomposition diminish the intensity of tetracycline fluorescence. The intensity of fluorescence varies with the analogues and follows the same order as their ability to cause discoloration. The decreasing order of fluorescence is as follows: LC, CC, DMTC, TC, PMTC, CTC, and OTC.

D. Dental Caries Protection?

Bevelander[147] interpreted Wallman and Hilton[148] as saying that the teeth of 50 children which demonstrated discoloration subsequent to tetracycline staining were highly susceptible to dental caries. Weyman and Porteous[144] reported the incidence of dental decay as similar to that in children with normal teeth unaffected by tetracyclines. Hennon[164] felt the dental caries incidence was not altered by tetracycline incorporation. Frankel and Hawes[163] and Frankel[175] stated that no significant association between dental caries and tooth discoloration was apparent. Anderson, Ferguson and Braude[113] felt that teeth stained with tetracycline would not be resistant to dental decay because bone incorporated with tetracycline did not remain bacteriostatic for more than four days. Swallow, DeHaller and Young[155] noted that in their relatively small population of cystic fibrosis patients with tetracycline discoloration, there was a

trend for a lower dental caries prevalence than a general population. Stephan et al in 1952[209] were the first to study CTC and other antibiotics in relation to dental caries prevention in rats. The antibiotics inhibited the formation of dental carious lesions. The antibiotics effective against gram positive bacteria inhibited dental decay the most, although not completely. Shaw and Sweeney in 1958[210] demonstrated that CTC and OTC were moderately effective in reducing dental caries in the cotton rat and white rat.

Larson and Zipkin in 1960[211] stated that tetracycline treatment may be an effective means of reducing dental caries activity if the bacteria which are eliminated by the antibiotic are not re-introduced. Zipkin, Larson, and Rall in 1960[212] and Zipkin and Larson in 1960[213] conducted studies in which dental caries in rats was markedly reduced by administering tetracycline. Larson and Zipkin in 1961[214] and Larson, Zipkin and Fitzgerald in 1963[215] stated that the reduction of dental caries caused by tetracycline was due to an altered bacterial flora in the rats which was not as cariogenic. Another study by Grahnen and Larson[216] found no significant difference in the incidence of tooth decay between premature and normal children. This fact is important when one notes that this controversy originated with the sample of Wallman and Hilton.[148]

Summary

Tetracycline incorporation does not seem to alter the tooth's resistence or susceptibility to dental caries. In animal experiments tetracycline lowered the dental caries incidence by altering the cariogenic flora. This lower incidence was maintained as long as the animals were not re-innoculated with the cariogenic strains of bacteria.

E. Tetracycline Induced Hypoplasia of Enamel

Wallman and Hilton[148,151] built a strong case that large doses of TC and OTC in premature infants were causing enamel hypoplasia. The most severe tooth changes were found with the highest total dose per kilogram of birthweight. The average dose was 210 mg./kg. with OTC and 189 mg./kg. with TC. Wallman[217] also reported a clinical observation that an infant who received TC immediately after birth had yellow teeth and the first and second primary molars were deformed with extremely sharp cusps. Witkop and Wolf[218] noted a high degree of correlation with TC staining and hypoplasia. Again, the higher the dose in these children the more damaging the result; these authors estimated that 21 to 26 mg./kg. will cause hypoplasia. Brown[159] and Beckelman and Gingold[3] reported cases of tetracycline hypoplasia. Brearley, Stragis and Storey[75] stated that 4.02 percent of those teeth stained with tetracycline also showed areas of hypoplasia. Baker and Storey[158] proposed that the incidence of tetracycline-associated hypoplasia in teeth at risk is approximately 26 percent. Demers et al[81] indicated that hypoplasia of tooth enamel and dentin could occur.

DeBorgarello and Gendelman[219] found under microscopic examination that enamel showing quantitative and qualitative alterations corresponded to areas of tetracycline fluorescence. There was a significant difference (P equals .001) in pigmented teeth displaying hypoplasia (23.79%) and non-pigmented teeth showing hypoplasia (2.44%). However, many other authors disagreed. Miller[153] believes that the hypoplasia that Wallman and Hilton referred to was in fact caused by prematurity and not tetracycline. Weyman and Porteous[144] felt that clinical hypoplasia could not

be related to the drug. Madison[59] found no evidence of enamel hypoplasia attributable to the drug. Swallow, DeHaller and Young[155] noted that only one patient out of sixty-three had tetracycline staining and hypoplastic defects. Harcourt, Johnson and Storey[181] noticed disturbances of dentin mineralization in the form of large interglobular areas but implied that these were not associated with tetracycline per se but were probably localized manifestations of chronic systemic illness.

Porter et al[220] ran a statistical analysis of 41 matched sets of full-term children in which one set received TC placentally and the other did not. Although there was staining of primary teeth, no statistically significant difference in hypoplasia was found in the TC group. Martin and Barnard[166] thought that any enamel opacities or hypoplasia was the result of the disease the drug was administered for and not the tetracyclines themselves. Mello[84] stated that there is insufficient evidence to show that tetracyclines are responsible for enamel hypoplasia when given in therapeutic doses during tooth development. Frankel and Hawes[163] said there was no significant association between tooth discoloration caused by tetracycline and hypoplastic defects in these teeth.

Grahnen and Larson[216] examined 68 premature and 61 normal-term children and found a significantly higher frequency of symmetrical enamel hypoplasia in the premature group, 2 percent to 21 percent. In the same study references to Gaunt and Irving[221] and to Lindquist and Rakit[222] were used to show that in animal experiments a deficiency of blood calcium causes severe disturbances in the calcification of teeth. An article by von Sydow[223] was cited as showing in the first days of life

premature children have lower blood calcium levels and a higher alkaline phosphatase levels in serum than full term children. Hamp[22] compared premature infants and found that those premature infants who received tetracycline had a higher incidence of enamel hypoplasia than those who did not receive the drug. Storey[179] took the middle of the road; although he did not deny that tetracyclines could cause hypoplasia, he wanted further evidence of a direct cause and effect between the drug and the structural abnormality. He felt as others had that children given large quantities of the drug are usually extremely ill, which in itself may be sufficient to affect formation of teeth. The answer, appears to lie between the extremes, and experimental data seemed to indicate that tetracyclines when administered to individuals with low serum calcium could cause hypoplasia. Baker[216] injected TC 250 mg./kg. intraperitoneally into rats which were parathyroidectomized and calcium deficient; he observed a rapid and prolonged fall in serum calcium following the injection.

Bevelander, Rolle and Cohlan[195] demonstrated an inhibition of calcification in rat teeth corresponding to tetracycline administration. Bevelander, Goldberg and Nakahara[224] demonstrated that tetracyclines in sufficient concentration could delay skeletal formation in sand dollars and that at higher concentrations they completely inhibited larval development. Concentrations which inhibited skeletal formation exerted their effect specifically at the onset of skeletal differentiation. Bevelander[146] in a later article stated that in teeth the larger the total dose relative to body weight, the more severe the abnormality. Johnson[183] confirmed that high dosages (200-250 mg./kg.) result in definite areas of enamel hypoplasia.

Nylen, Omnell and Löfgren[206] found pronounced hypoplastic and hypomineralized lesions in the enamel laid down during the full period of drug administration, while enamel formed before and after appeared normal. They said that the primary effect of tetracycline seems to be ameloblastic impairment leading to the formation of hypoplastic enamel and that impaired cells probably allow passage of the tetracycline since only the affected enamel is labelled with fluorescing tetracycline. In a later study Löfgren, Omnell and Nylen[199] found little effect on the enamel when TC and OTC were injected in low dosages. In contrast, high dosages of both TC (150 mg./kg.) and OTC (154 mg./kg.) resulted in developmental disturbances ranging from an incremental band only to one that included a gross hypoplastic lesion. A comparison between the two drugs revealed a much higher incidence of hypoplasia among the defects caused by TC than those due to OTC.

McIntosh and Storey[180] found that in rats 50 mg./kg. caused discoloration; 100 mg./kg. discolored and caused a decreased thickness in the enamel with some aplasia of enamel showing dentin in some areas; and that 200 mg./kg. caused severe aplasia of enamel. They also found that the different analogues had effects of varying severity for hypoplasia, they were in decreasing order, EAHTC, DMTC, LC, TC>CTC, OTC, MTC>DTC, AHTC. An oral dose had to be four or five times the intraperitoneal dose to produce the same effect. Saxen[225] developed an *in vitro* test for calcification inhibition of the analogues. She found that different analogues behaved differently at different concentrations. At 1 microgram/ml. she listed in decreasing order TC I, DMTC, TC II, MTC, CTC and OTC; at five

micrograms/ml. DMTC, TC II, TC I, MTC, OTC, and CTC; and at twenty micrograms/ml. TC I equaled TC II, MTC, DMTC, CTC, and OTC.

Yen and Shaw[226] indicated in their study that 4 mg./kg. of oral minocycline caused no apparent effect on dentin apposition. Walters and Sayegh[42] found that 2 mg./kg. of minocycline intraperitoneally produced less fluorescence than 25 mg./kg. of OTC or TC given the same way. The initial microradiographic picture of the fluorescent areas in bone and dentin was suggestive of hypoplasia. Grøn and Johannessen[204] examined ground sections of teeth from rats which had been injected with 100 mg./kg. of OTC; the fluorescent bands in dentin corresponding to the time of injection were hypomineralized when the sections were submitted to microradiographs. Moffitt[156] found that dentin microhardness was higher in non-stained (non-tetracycline and without yellow fluorescence) areas than in stained areas, (P equals 0.03). Antalovska and Beran[173] found that dental tissues are usually less mineralized in places which show tetracycline fluorescence. Brázda, Kolc and Zastava[172] found that high doses of tetracycline given during intensive formation of the enamel result in enamel hypoplasia. Owen[145] and Bennett and Law[186] identified areas of hypoplasia in the enamel of dogs who received tetracycline.

As stated earlier, Thanik and McMurchy[141] showed cessation of growth in rats given 80 mg./kg. of CTC or DMTC intraperitoneally, and OTC was the most inhibiting at very high dosage. Cohlan, Bevelander and Tiamsic[136] conducted a study concerning tetracycline administration to premature infants. They concluded that a forty percent depression of normal skeletal growth had occurred as measured by inhibition of fibula growth. Fibula growth inhibition was rapidly reversible after cessation

of the tetracycline. They also found that 40 mg./kg./day of TC during the tenth through the fifteenth day of gestation in rats resulted in a 26 percent reduction in expected fetal size at term. However, Johnson and Mitchell[43] found that a study group of young growing rats with limited oral dosages of tetracycline apparently had no significant effect on either weight gain or femur growth after 39 days.

Summary

Tetracyclines do have the potential to alter growth. In high dosages during periods of lowered serum calcium tetracyclines can increase the amount of enamel hypoplasia that would otherwise be seen in the developing teeth.

F. Possible Binding Mechanisms of Tetracyclines

The exact mechanism of tetracycline incorporation into mineralizing tissue is not clearly understood. Regena et al[33] in 1951 said that OTC chelated to calcium ions; Albert[35] demonstrated this effect with many metallic cations for both OTC and CTC in 1953.

In 1957 and 1958 Milch, Rall and Tobie[65,66] stated that it may be surmised, if only on the basis of the lack of persistence of fluorescent material in tissues richly supplied by body fluids, as in the reticuloendothelial system, that mere availability of the administered analogue is not the sole mechanism involved in its persistence in osseous tissue. They noted that tetracycline localization in bone appeared to be limited to areas of new bone formation and suggested that bone fluorescence following tetracycline administration may be attributed to the binding of either the unaltered compound or metabolic derivative to calcium and/or the matrix of newly formed bone.

Loo, Titis and Rall in 1957[227] presented evidence that tetracycline is unchanged and bound as a loose complex to a peptide in mouse sarcoma tissue. Titus, Loo and Rall[228] in the same year reported that the binding of tetracyclines to bone structure is dependent on pH and possibly involves metallic cations either to the inorganic structure or to the organic matrix. Häkkinen[229] in 1958 confirmed that calcium ions are involved with the binding of tetracyclines in experimentally produced metastatic tumors in rats. Malck and Kolc[230] found that maximum absorption of CTC in tumors occurred in areas affected by calcification.

Plaza-Roca[110] noticed that aqueous solutions of OTC did not fluoresce but when they were passed through filter paper the filter paper fluoresced. This was the same fluorescence exhibited by the dry OTC and was seen where serum had oozed on the casts of patients receiving OTC. Plaza-Roca believed that the tetracyclines were linked to basic bone substance through the action of mucopolysaccharides at the time that calcium is laid down.

Buyske, Eisner and Kelly in 1960[231] noted that TC decline from bone is faster than for CTC; they attributed this to the fact that TC is not as good a chelating agent as CTC. They also demonstrated that physical contact of the antibiotic with bone immediately produced attachment. This was revealed by removing a rat femur and stirring it for one minute in a solution containing twenty micrograms/ml. of tetracycline, and discovering that the bone took up the tetracycline fluorescence which could not be washed off under running water.

Frost and Villanueva[232] said in 1960 that tetracycline was merely "cemented" in by further mineralization since with fresh bone <u>in vitro</u>

they were able to stain and destain bone surfaces of all types at will. Hattner and Frost[233] in 1962 discovered that the fluorescence of tetracycline molecules is quenched in water and methanol but not in carbon tetrachloride, indicating that a dipole action is involved. They further stated that the fluorescence of tetracycline molecules fixed in mineralized bone is in part the result of loss of hydration shell. The loss is probably related to the increasing amounts of mineral phase with increasing time which characterizes all new bone *in vivo*; of major importance in the fixation of tetracyclines to mineralizing bone is the steric placement of carbonyl and hydroxyl groups in the apatite lattice.

Due to the fact that tetracycline-induced fluorescence localizes only in those areas where matrix calcification was observed, Milch, Tobie and Robinson[234] postulated that tetracyclines bind to calcium of "seeded" crystal nucleation sites and their immediate derivatives on collagen fibrils presumably via the oxygen atoms of the D-ring of the naphthacenecaboxamide nuclei.

Kelly and Buyske[92] demonstrated that except for metal chelate formation, tetracycline was chemically unaltered in the rat and the dog; therefore, no metabolic transformation had occurred.

Harcourt, Johnson, and Storey in 1962[181] noted that dentin stains and enamel does not appear to stain; therefore, tetracyclines must not complex to calcium cations and must be binding to organic matter rather than inorganic matter. They proposed the hypothesis that a complex of ground substance, collagen and mineral may be the mechanism by which tetracycline is bound. Davis, Little and Aherne[130] suggested that TC is deposited on the organic matrix of bones and teeth—gave no factual substantiation for this opinion.

Hilton[111] showed that when calcium orthophosphate was precipitated from aqueous solution which contained tetracycline hydrochloride, the tetracycline was adsorbed onto the precipitate.

Ibsen and Urist[235] in 1962 published the first of several articles dealing with tetracycline and binding. They showed that calcium and magnesium binding by OTC, observed spectrophotometrically, occurs in a step-wise fashion. Higher metal to ligand complexes may exist but the 1:1 and 2:1 OTC complexes are postulated to be the chief molecular species occurring in vivo. They postulated that when 1:1 or higher calcium to OTC complexes were formed it was unlikely that all coordinating positions of calcium were satisfied. Apatite surface calcium could react with OTC and permit accumulation of the fluorophore in bone. Possibly, it may be that spatial configuration of apatite surface calcium allows two or more calcium atoms to simultaneously bind one molecule of OTC, enhancing the force of attraction. Urist et al[236] described their method using frog heart muscle in which the calcium complexes of OTC were analyzed.

In 1963 Urist and Ibsen[188] stated that the binding of OTC by calcium salts in vitro depends upon the formation of a complex with calcium ions in the surface of the microcrystals of apatite. The large OTC molecule presumably occupies a crystal surface position comparable to that of citrate, carbonate, and other ions. If OTC binding is a function of calcium ions in the surfaces of crystallites, the mechanism can be that of chemisorption, which refers to electrovalent or covalent bonding; the chemisorped OTC is limited to a surface monolayer. It may also involve ion exchange. Thus, the bone mineral behaves like an ion exchange column in which OTC may exchange for H^+, $CO_3^{=}$, or OH^- or citrate$^{\equiv}$ ions.

Urist and McLean[237] in 1963 outlined three possible binding mechanisms for tetracyclines. These were (A) in the crystal surfaces as complexes with calcium, (B) as complexes with collagen or (C) in complexes that share calcium ions with polysaccharides in newly mineralized tissue. Diagrammatically they are:

A	B	C
Collagen	Collagen	Collagen
•	|	•
Polysaccharide	OTC	Polysaccharide
•	•	|
Apatite	Polysaccharide	OTC
|	•	|
OTC	Apatite	Apatite

Hypothesis (A) was supported as the best of these, since evidence was presented that OTC is bound by apatite crystals in aqueous solution and by inorganic bone. These authors contend that all substances which bind OTC have calcium ions (gastric mucosa, scar tissue, cornea) and that the small crystal size, high reactivity, and large amounts of fluid in fast-growing bone predispose it to OTC uptake. Older bone is inert due to the large crystal size and the denseness of matrix which prevents free diffusion of the large OTC molecule. Hypothesis (B) is difficult to prove since collagen binds only relatively small quantities in vitro and it cannot be divested of all mucopolysaccharide and metal ions that form complexes with tetracycline. Although (C) is a possibility, direct chemical evidence is lacking.

In 1964 Ibsen and Urist[36] admitted in another article that tetracyclines form complexes with organic as well as inorganic compounds, but the inorganic compounds have the characteristics of chelates and appear to be more stable. Bone-bound tetracycline shows polarized fluorescence;

fluorogens in solution do not emit polarized fluorescence, while organized crystals of fluorophores do, this should rule out binding to polysaccharides. Soft tissue binding does occur and is due almost certainly to tetracycline complexes with organic molecules stabilized by metal ions or perhaps by lipids. The authors hypothesized that such stabilization may be due to partial dehydration of the tetracycline molecule--breaking intramolecular hydrates and freeing reactive centers.

Finerman and Milch[238] in 1963 presented evidence which agreed with Ibsen and Urist in that tetracycline binds to the calcium ion in tissue. They stated that this was because decalcified bone would not take up tetracycline and deproteinized would (in vitro) and concluded that tetracycline probably interacts, primarily if not exclusively with calcium ions, at least in hydroxyapatite seeded nucleation sites on collagen fibrils.

However, Deleu[239] stated in the same year that CTC was attaching to organic tissue as CTC localized in the crystalloid membrane of the eye. Prochazka et al[240] in 1964 showed that the lasting tetracycline fluorescence in the islets of Langerhans in the human pancreas was due to the formation of a complex of CTC with insulin mitigated by a bivalent cation of zinc.

Owen[145] in 1964 stated that if binding of tetracycline antibiotics is a function of the chelating ability, the strongest chelating agent should give the deepest coloring. His experiments seemed to support this premise.

Johnson in 1964[183] found that the tetracycline distribution in dentin is apparently related to the distribution of the mineral phase and follows well known features of dentinogenesis. In enamel there appears to be an initial distribution related to the organic phase and a secondary distri-

bution related to the mineral phase during maturation of the preformed matrix. TC uptake by enamel is greatest where the mineral content is lowest, since it diffuses rapidly through the highly permeable immature matrix. This fact per se does not indicate whether the drug is associated with the mineral or organic phase or both. The fading during progressive mineralization may be due to removal of the drug from the matrix, along with large amounts of water, protein, and mucopolysaccharides, which occurs at this stage. However, it could equally well be due to the masking of the fluorescence by additional influx of apatite crystallites. Probably two distinct processes are involved and that tetracyclines chelate both the inorganic and organic substances.

Zastava et al in 1964[90] confirmed other reports that the TC molecule is mediated by the calcium cation, but fixation through other cations is also possible.

Mulvaney, Beck and Qureshi[91] in 1964 found that OTC binds only to reactive crystals and through aging the crystals of apatite no longer readily accept OTC in urinary calculi.

Bevelander in 1964[146] and Bevelander and Nakahara in 1965[174] were unable to exclude the possibility that the fluorophore may combine with the organic matrix of dentin and enamel as well as the mineral component.

Epker[241] presented cases in 1966 in which the zone of mineralization in dentin was precisely labeled by tetracycline. He used this as evidence that tetracyclines combine with the mineralizing phase of dentin and not with the organic matrix of the dentinoid per se.

Eger, Gattow and Kammerer[242] gave evidence on how tetracyclines derange mineralization and osteogenesis. They presented this in a step-wise

fashion. A) Tetracycline molecules are incorporated into crystallite surfaces of apatite and octacalcium orthophosphate. B) The calcium ions on the surface are saturated as regards to coordination number and growth of the crystallites is blocked. Moreover, under certain conditions the formation of crystal nuclei is prevented by the presence of fully complexed calcium ions. C) The hydrolysis of octacalcium orthophosphate into apatite is impeded or even stopped by chelation of the crystal surface. The surface of these partial tetracycline--calcium ion complexes is hydrophobic and for reason of lattice dynamics their dissociation in aqueous solvents is meager. Octacalcium orthophosphate must therefore accumulate in tetracycline-labeled bone and this would explain why such bone yield more pyrophosphate on heating to 325°C than normal bone.

Bennett and Law[186] and Bennett[187] found supporting evidence for the theory that tetracycline combines with the surface calcium ions of the apatite crystal when their analysis of enamel and dentin showed that tetracycline bound to dentin and enamel in a ratio of 9 to 1.

Hammarström[198] in 1967 showed that shortly after injection, both tetracycline and Ca^{45} accumulated in a superficial zone in newly-deposited enamel matrix. In addition, Ca^{45} was taken up throughout the whole thickness of other areas of enamel with no corresponding accumulation of tetracycline. This uptake of Ca^{45} was localized occlusally of the superficial zone. Between these two areas there was a superficial zone of increased fluorescence and autoradiographic blackening. Both substances accumulated in the developing enamel, but there were great differences in their distributions. The distribution of Ca^{45} supports

the theory of two stages in the mineralization, and tetracycline seemed mainly associated with the primary stage.

A physicist, Kallmann,[243] in 1968 considered Terramycin and hypothesized that the yellow fluorescence of tetracycline from ultraviolet light in living tissue is a process of surface adsorption of the drug. Subsequent tissue growth locks the drug into bone or other living tissue.

Kawasaki[244] in 1972 presented evidence that the binding of tetracycline occurs in both the organic and inorganic phase of dentin. He injected piglets with TC, tetracycline was deposited in the dentin, and the in vivo fluorescence could be regenerated after fixation and decalcification or after removal of the organic matrix by placing the specimens in a solution containing tetracycline.

Summary

The exact mechanism of tetracycline deposition and fixation in bone and teeth still remains unclear and additional research is necessary. The mechanism which seems to be the most feasible is a chemisorption of the tetracycline molecule to the surface of the apatite crystal mediated by calcium cations.

G. Tetracyclines: Uses in Research

The fluorescence of tetracyclines has been used to great advantage in many divergent areas of research as a vital dye.

Initially the pharmacology of the tetracyclines themselves was studied. Andre,[63] Milch, Rall and Tobie,[66] Helander and Böttiger,[64] and Böttiger[67] traced the distribution of tetracycline by means of fluorescence.

Frost[207,245] used tetracycline labelling to study the thickness of osteoid mineralization per day and the half-life of some human bones.

Boyne,[246,138] Boyne and Kruger[247] developed the use of tetracycline as a vital dye in dental surgery for study of the healing of fractures, for characterization and correlation of various osseous repair responses of the traumatized host in various anatomical loci, for bone graft materials, and for surgical correction of malocclusions.

Gregg and Avery[248] used TC as a label for vital bone in the alveolous to determine growth of the alveolar bone.

Cleau, Perkins and Gilda[137] and Yen and Shaw[226] found that tetracycline was an excellent vital stain for demonstrating calcifying bone or tooth structure.

Tobie and Beye[96] used tetracycline to locate and visualize migrating and subcutaneous filarial worms.

Johnson[21] and Hennon[164] proposed that tetracyclines could be used to compare the sealing capabilities of various restorative materials in dentistry.

Mulvaney, Beck and Qureshi[91] proposed "tagging" urinary calculi by administering a tetracycline and examining the ring-growth to determine the history of the stone.

Perhaps the most exciting developments in the late 1950's was the belief by many authors that tetracyclines preferentially deposited in malignant tumors. Rall et al,[249] Hakkinen and Hartiala[250] and Mcleay and Walske[112] all noted this and thought that the drug could be used to detect and locate malignant tissue. Berk and Kantor[251,252] and Klinger and Katz[253] even devised a method of pretreatment for patients with suspected gastric cancer and reported a high degree of correlation with those ulcers that fluoresced and their malignancy.

However, Ackerman[254] wrote that studies in man and experimental animals demonstrated that tetracycline localization in malignant tissues was erratic and undependable. Tetracycline was often seen in non-cancerous, necrotic, inflamed and calcific lesions. Therefore, he warned that these limitations should be realized when using the drug for cancer diagnosis. Mustakellio[152] stated that tetracycline fluorescence is not an indicator of malignancy but merely an expression of stromal reaction favoring calcification.

Malek[255] provided an overview of the use of this group of drugs for research in areas other than antimicrobial. He stated that the fixation of tetracycline is not a specific property of some cells, tissues or pathological conditions. It can be found in very divergent pathological conditions--lipid necrosis in pancreatitis, in tumors, in retention of burnt skin and in damaged myocardial muscle.

Summary

The use of tetracycline in research other than antimicrobial is generally confined to its use as a vital dye to determine hard tissue growth and development.

III. Bleaching of Teeth

A. Non-Vital Teeth

Pearson[256] in 1958 described his method for bleaching a stained non-vital endodontically treated tooth. After placing a rubber dam on the tooth in question, he desiccated and cleaned it with a 1:2 mixture of 95 percent ethyl alcohol and chloroform. The tooth was then pumiced. A

bleaching solution of 75 percent ether and 25 percent hydrogen peroxide was placed on the tooth. This mixture was activated by a photo-flood lamp. The author claimed that the actinic radiation plus heat was necessary for a good bleach--heat alone was not enough. The lamp was a number two photo-flood placed about twenty inches from the tooth. The treatment usually lasted about twenty minutes. Then, after the final bleach, he sealed both the enamel and dentin with self-curing acrylic monomer to prevent extrinsic stains from entering the tooth.

Spasser[257] in 1961 described another method for bleaching non-vital teeth. He used a solvent to remove oil residue after the pulp chamber of the endodontically treated and sealed tooth had been cleaned. Then a creamy mix of sodium perborate and water was placed in the chamber and sealed in the tooth for about four days. This procedure was repeated three or four times until the proper shade of tooth color was obtained. Spasser could not predict how long the tooth would maintain the new shade since the stability of the bleached tooth shade was determined by external enamel cracks and the integrity of the marginal seal of the restoration.

Nutting and Poe[258] introduced an innovative approach with the "walking bleach" technique. This method bleached endodontically treated teeth by sealing in a paste of sodium perborate and superoxol (35 percent hydrogen peroxide). This paste was periodically replaced until the desired tooth color was obtained. These authors felt that their procedure was clinically effective, simpler and less time consuming than the heating methods.

In 1967 Nutting and Poe[259] restated their method of sealing sodium perborate and superoxol in the pulp chamber of endodontically treated

teeth and after a successful bleach, restoring the pulp chamber with silicate cement. Also, they mentioned that sodium perborate monohydrate (Amosan) could be used.

Serene[260] in 1973 referred to the use of 30 percent hydrogen peroxide and sodium perborate mixed as a thick paste and sealed into the pulp chamber. He believed that it was extremely important to seal the root canal filling at the base of the pulp chamber with a mixture of zinc oxide eugenol to prevent the bleaching agent from entering the root canal. He also advocated a final seal after the bleaching agent was removed by placing restorations of silicate cement covered with gold foil.

Stewart[261] in 1965 isolated the discolored tooth with rubber dam. Cotton pellets saturated with 35 percent hydrogen peroxide were inserted into the clean pulp chamber and the pellets warmed by applying an endodontic drying point until the hydrogen peroxide began to "boil."

Caldwell[262] described the use of a comparatively new instrument* as a heat source for bleaching discolored teeth or preventing discoloration after endodontic treatment. The technique was as follows: 1) Plug the heating instrument into a 110 volt system and allow 3 to 4 minutes for the instrument to reach working temperature. 2) Isolate the tooth or teeth with a rubber dam. 3) Remove all restorations from the tooth or teeth to be bleached and root canal filling material to a point 2 mm. apical to the gingival margin. 4) Flush the tooth with a desired irrigating solution and dry thoroughly. 5) Place saturated cotton pellets of 35 percent hydrogen peroxide in the cavities, in the pulp chamber, and over the crown of the tooth. 6) Have the patient warm the 35 percent

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* Bleaching Tool, Fluor-Ted Co., Inc., Davis, California

hydrogen peroxide by holding the heating instrument against the cotton pellets on the tooth with slight pressure. If the heat becomes uncomfortable for the patient, they are instructed to remove the instrument for a few minutes. The hydrogen peroxide is replenished as the cotton pellets dry. Most appointments will last approximately thirty to forty-five minutes. By heating concentrated hydrogen peroxide to 165°F, the bleaching rate of the drug will be increased about 200 times.

B. Vital Teeth

1. Fluorosed Teeth

McInnes[263] in 1966 described the following method which he had used for twenty years to improve the esthetics of fluorosed teeth. 1) The teeth were cleaned with pumice and isolated with a rubber dam. 2) The teeth were wetted for 15 to 30 minutes with a solution of 5 parts 30 percent hydrogen peroxide, 5 parts 36 percent hydrochloric acid, and 1 part ether. 3) The solution was neutralized with a mixture of baking soda and distilled water. 4) The dam was removed and the teeth polished with cuttlefish discs and moistened pumice.

Bailey and Christen[264] in 1968 stated that endemic dental fluorosis is a common clinical entity, especially in patients living in rural areas of southwestern United States. As a safe, practical method of stain removal, they used a solution of 5 parts 30 percent hydrogen peroxide, 5 parts 36 percent hydrochloric acid, and 1 part anesthetic ether, along with light discing of the tooth surfaces. Fourteen patients were treated with marked success in every case and no adverse sequelae. The two

authors cautioned that only teeth with a smooth, marbled appearance should be chosen for the bleaching procedure; deep hypoplastic areas were contraindicated, as were teeth with paper-white areas or tetracycline-stained teeth.

Bailey and Christen[265] conducted a test under in vitro conditions to determine whether the previously outlined technique by McInnes destroys excessive amounts of tooth structure. A standarized bleaching process by McInnes was applied for 20 minutes to the labial surfaces of 27 extracted permanent maxillary anterior teeth with endemic dental fluorosis and to 27 control teeth. The enamel thickness on the labial of each tooth was measured before and after treatment. The authors found that the enamel removed was constant for both fluorosed and normal teeth; it was less than 20 percent, approximately 0.1 mm. for each twenty minute treatment, for 92 percent of the specimens. There was no statistically significant difference between fluorosed and non-fluorosed teeth in terms of thickness of labial enamel.

Colon in 1973[266] also used the McInnes technique for removal of severe endemic dental fluorosis: he used the same solution for two 15 minute appointments one week apart. He had results comparable to those of others using this technique.

Bouschor[267] in 1973 used superoxol and heat to bleach teeth with fluorosis. He isolated the teeth with rubber dam and warmed a mixture of 30 percent hydrogen peroxide and ether held against the teeth in saturated cotton rolls. The solution over each tooth was warmed for three or four seconds rotating from tooth to tooth for 20 to 30 minutes. Four such treatments were usually required.

2. Tetracycline-Stained Teeth

Cohen and Parkins in 1970[5] achieved significant esthetic improvement in five of six patients with tetracycline staining of their teeth by bleaching these teeth with 30 percent hydrogen peroxide heated to 88°F with a hand-held heating instrument for thirty minutes. Each patient was given eight treatments, generally at one week intervals, and was subsequently evaluated at monthly visits. The authors observed that the thinness of enamel of the lateral incisors as compared to the central incisors and cuspids accounted for the most dramatic shade improvement of the lateral incisors. They also stated that after the rubber dam was removed the teeth appeared chalky white for one hour. The long term results are still under observation and to be reported on.

Arens, Rich and Healey in 1972[6] and Rich[268] reported the results of five patients having tetracycline-stained teeth who were bleached with 35 percent hydrogen peroxide and heat. The study demonstrated that a good esthetic result can be achieved by bleaching tetracycline-stained teeth. Bleaching was accomplished by heating 35 percent hydrogen peroxide saturated in cotton rolls and held against the teeth with a hand-held instrument at 10°F less than the highest temperature at which discomfort was first illicited in the patient's tooth. Five patients from 7 to 16 years of age were treated. Yellow and yellow-brown stains were more easily and completely removed than was gray stain. The incisal one-half of the teeth bleached better than the cervical half. Vitality tests before and after showed no measurable change in the teeth. When treatment was completed, the teeth no longer fluoresced under ultraviolet light.

Of the five patients, three showed a marked improvement and two a slight improvement. The patients were treated for three 20 minute sessions at weekly intervals.

C. Considerations in Bleaching Teeth

Nyborg and Brannstrom in 1968[269] histologically examined teeth after the application of a heating instrument at 150°C for 30 seconds and reported very little cellular infiltration in 6 pulps and none in 14.

Mumford in 1966[270] showed that when teeth are isolated by rubber dam the enamel becomes whiter, but regains its normal appearance when re-exposed to saliva. This was thought to be due to the loss of moisture from the enamel and subsequent re-imbibing of moisture from the saliva.

Another consideration in the bleaching of teeth is the effect of the heating instrument on the enamel and the additional staining that may occur if the instrument causes cracks in the enamel. Peultier, Frank and Klewansky[271] in 1967 found that sudden changes of temperature applied during a varied time period was the major factor in production of enamel cracks. Variations from normal to cold (21°C) were more harmful than from normal to warm (60°C). Alternating between 21°C to 60°C produced enamel cracks more easily in younger age groups. The authors also showed that a hot needle used in cautery as well as ethyl chloride produced enamel cracks.

Wainwright and Lemoine[272] in 1950, using urea radioactively labeled, and Bartelstone in 1950[273] and 1951,[274] using I-131, found that a diffuse penetration of enamel occurs without the necessity of following lamellae or cracks. Penetration of the isotopes through enamel into dentin was

more frequent near the gingival line and near the occlusal fissures. In several teeth the radioactive material was traced continuously from the grossly intact non-carious enamel and dentin to the pulp.

In summary, the literature on bleaching teeth is empirically directed. Further investigation is needed of the exact mechanism involved in altering tooth color.

IV. Fluorescent Photography

Fluorescent photography of living subjects in color is a powerful diagnostic tool. Many pathologic conditions appear differently under ultraviolet light. Thus, the pathological state can be easily differentiated from the normal.[275]

Two methods have been used to obtain fluorescent photographs of subjects. The first, uses a continuously emitting source of long-wave ultraviolet light and very long exposure times. Adams[276] used a camera ratio of 1:1 with an F-stop of 8, a Kodak 2B barrier filter, Kodachrome II film and a three minute exposure to obtain his fluorescent photograph. The second method which is more acceptable to the patient is to use a Wratten 47-A or a Corning Glass filter 5970 over a 200 watt-second flash source for generating flash ultraviolet light. Hi-Speed Ektachrome film is used with an F-stop of 2.8 to 8. Barrier filters are often used to enhance the contrast. The flash fluorescent photographs have the advantage of being more reproducible and require a fraction of the exposure time.[79,190,205,275,277]

V. Television-Electronics in Dental Research

Using the television microscope, Klein,[278] described a technique and instrumentation for intraoral clinical investigation. His initial work was followed by several other investigations.[279-284] In 1967 Klein and MacPherson[285] described electronic equipment which could measure distances, in microns, on the intraoral radiograph. The distances are measured with a series of calibrated dots which are superimposed on a television scan line. Further investigation by these authors[286] in photographing stored television microscope images led to sophisticated instrumentation that could store and measure a series of video images. A later report by Klein[287] dealt with intraoral microscopy, linear-density measurements of radiographs, and subtraction radiography. The use of television-electronics in dental research is in its infancy. Time should prove it an invaluable adjunct for the dental investigator.

METHODS AND MATERIALS

Twenty-two male New Zealand white rabbits, each weighing three to four pounds, were selected for the investigation. The animals were caged individually in a room maintained at 70°F with 12 hours light and 12 hours darkness. The cages were cleaned every three days and once a week all cages were sterilized. Every three days the rabbits were weighed to the nearest tenth of a pound. Their diet consisted of Purina Rabbit Chow* given ad libitum.

The rabbits were divided into two groups and assigned an identification number by using a random numbers table.[288] To ensure that the animals would remain in their assigned group, the right ear of each rabbit was tattooed with the identification number. Seven rabbits received distilled drinking water and a subcutaneous injection of 0.10 ml. of isotonic saline per pound of body weight every three days. Fifteen rabbits received 5.0 mg. of oxytetracycline** per pound of body weight in divided doses twice a day in their drinking water and 5.0 mg. of oxytetracycline*** subcutaneously every three days. The rabbits were maintained on this regimen for eight weeks and all incisor teeth were checked with an ultraviolet lamp**** every two weeks for evidence of tetracycline fluorescence. Administration of oxytetracycline was discontinued the day before the clinical bleaching of the incisors, and thereafter all animals were given distilled drinking water.

*Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri
**Terramycin Pediatric Syrup lot No. 1Y493, Pfizer Laboratories, New York, N.Y.
***Terramycin Intramuscular lot No. 8Y911, Pfizer Laboratories, New York, N.Y.
****Blak Ray B-100 A, Ultra Violet Corporation, San Gabrial, California

Clinical Bleaching

Of the 22 rabbits used in the study, three control animals which were not to receive the drug accidentally received OTC and were destroyed. The remaining 19 animals were randomized into five groups as follows: Three rabbits with tetracycline-stained teeth were not bleached and were used to determine if incisors in the same arch stained equally, and could be used as their own controls for bleached and unbleached stained teeth. Four rabbits without tetracycline staining would be bleached to determine if the bleaching process affected the teeth; two of these would be bleached once, the other two twice. Twelve rabbits with tetracycline-stained incisors would be bleached; four would be bleached once, the remaining eight would be bleached twice. In all cases of bleaching, only one incisor in each arch was bleached and the other incisor acted as the unbleached control.

The rabbits fasted 24 hours to prevent regurgitation of stomach contents during anesthesia. The animals were injected into an ear vein with 15.0 mg. of Nembutal* per pound of body weight. The incisor teeth were photographed with a Minolta SRT-101 camera using a 100 mm. short mount lens on an automatic bellows, a Tiffon 81-A filter and a Braun F-111 strobe-light. Kodachrome X film at 1/60 of a second and an F-stop of 32 was used. A fluorescent photograph was then taken using the same basic camera system, but it was modified slightly by placing a Kodak Wratten 2B barrier filter in front of the lens system. The incisors were illuminated in the darkened room with only long-wave ultraviolet

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*Nembutal Sodium Solution, Abbott Laboratories, Chicago, Illinois

light from a 100 watt mercury vapor lamp.* The camera, light source, and rabbits were kept in a constant relationship and using an F-stop of 5.6, a one-second exposure was made. All slides were taken at a 1:1 image to film ratio. A pre-operative and post-operative slide in white and ultra-violet light was taken during each bleach. A one-day post-operative slide in white light was taken after the final bleach and before the animal was sacrificed.

The incisors were given a five second per tooth dental prophylaxis using flour of pumice moistened with water and applied with a rubber cup and slow speed handpiece. An attempt was made to match the four incisors with a shade guide.** An extra-heavy rubber dam was applied first to the left maxillary incisor, then to the mandibular right incisor, and each was bleached for 10 minutes. The dam was held in place by an Ash number nine clamp and a frame constructed of 0.040 stainless steel wire spot-welded together (Figures 1 and 2).

Thirty percent hydrogen peroxide*** was held on the isolated tooth with saturated cotton pellets and a stop-watch was used to time the application of heat to them. Intermittent application of heat was supplied by alternating the heating element**** for 30 seconds on the labial surface and 10 seconds on the lingual surface for a total time of 10 minutes. The temperature of the heating element was maintained at 110°F and gauged periodically with a thermometer.

*Blak Ray B-100 A, Ultra Violet Corporation, San Gabrial, California
**Trubyte Biotone Shade Guide, Dentsply International, Inc., York, Pa.
**Sevriton Simplified Shade Guide, Claudius Ash Co., London, England
***Hydrogen Peroxide 30% C.P., J. T. Baker Chemical Co., Philipsburg, N. J.
****Indiana University-Union Broach Bleaching Instrument, Union Broach Co., Long Island City, New York

Twenty-four hours after the bleaching treatments were completed, the animals were sacrificed by guillotine and the clinical crowns of the incisors were removed at the level of the gingiva with a high-speed carbide bur. The lingual of each incisor was scored for later orientation of the ground section on the microscopic glass slide. Throughout the preparation of the ground sections the specimens were stored in a laboratory drawer of a darkened laboratory maintained at 60°F.

Preparation of Ground Sections

The teeth were coded and placed in separate vials of 10 percent formalin for 24 hours. Next the specimens were dehydrated on successive days in alcohol in concentrations of 70, 80, 95, 100, and 100 percent ethyl alcohol. The teeth were then placed in styrene for 24 hours, followed by a day in an equal mixture of Bio-Plastic* and styrene. The next day the teeth were embedded in Bio-Plastic with their code number and allowed to cure for one week. The specimen blocks were then removed from their molds and polished with wet pumice and a cloth wheel on a lathe. The polished blocks were mounted on acrylic sectioning jigs. Serial transverse and approximately plano-parallel ground sections were prepared on a thin sectioning machine** (Figure 3) at 100 ± 5 microns, and checked on a stage micrometer.*** The teeth were arbitrarily divided into thirds representing incisal, middle and gingival thirds of the tooth. Each third produced about 4 ground sections. The ground sections

*Ward's Bio-Plastic, Ward's Natural Science Establishment, Inc., Rochester New York

**Gillings-Hamco Model GH-1, Hamco Machines, Inc., Rochester, New York

***Federal Products Corp. Model P-31, Providence, Rhode Island.

from the contralateral teeth for a specific third were mounted in pairs on the same 1 inch by 3 inch glass slide with Histoclad* and cover-slipped with a number one Corning cover glass. All slides were stored in a light tight box at 40°F in a refrigerator until they were examined.

An independent investigator selected the most ideal ground section from the incisal, middle and gingival third of each tooth for measurement. His selection was founded on identifying the ground section in each third closest to being plano-parallel and 100 microns thick. These sections were then coded to prevent identification of bleached and unbleached specimens before measurement. This eliminated bias on measuring the fluorescent intensity of the specimens; however, it meant that the sections selected were not necessarily from identical positions in that third of the pair of teeth compared.

Fluorescent Intensity and Linear Measurements

All electronic linear and amplitude measurements of tetracycline fluorescent intensity were completed in the Television and Electronic Dental Research Laboratory of Indiana University School of Dentistry.

The television microscope measurement instrumentation was developed by Klein and MacPherson.[285,286] An ultraviolet light microscope** was coupled to a closed-circuit color television system with scan-line measurement circuitry (Figures 4 and 5). The microscope was adjusted for fluorescent microscopy using a planapo 4/0.16 objective mounted in

*Histoclad, a low fluorescing synthetic mounting medium, Clay Adams, Parsippy, New Jersey

**Zeiss Large Universal Research Microscope, Carl Zeiss, West Germany

a deflector FL assembly with a 53 insert barrier filter. The light source was a special purpose illuminator with a HBO-200 watt super pressure mercury lamp with BG-12 and BG-38 exciter filters. The lamp's power supply was stablized with transformers supplying a constant line voltage of 105 volts. This arrangement provided a televised image of 2.5 mm. of the tooth specimen under measurement.

The color television camera* coupled to the microscope provided video signals from the red channel only. This channel provided the best video signal of the fluorescent image, but the signal was still weak and required amplification with a video distribution amplifier. The amplified signals were passed through a narrow pass filter to reduce the electronic noise caused by insufficient light from the fluorescing image. The processed signal was then applied to a calibrated differential comparator (Tektronic type z plug in unit) mounted in the oscilloscope.** The comparison voltage control readout was utilized to position the video signal's wave-form at a reference level on the oscilloscope graticule and provide a digital reading of the amplitude of fluorescent intensity.

The linear measurement system identified the position and distance of each fluorescing band of tetracycline from the outer enamel surface. The measurement system enabled the operator to select, identify, and illuminate any one of the 525 lines of scan of the televised fluorescent image, and generate a marker dot that was moved along the identified and illuminated line by a vernier control.

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*Shibaden Model HV-1100 U, Shibaden Company, Chicago, Illinois.
**Tektronix Model RM 35 A, Tektronix, Inc., Portland, Oregon.

Calibration of the instrumentation was accomplished by focusing the microscope assembly on a Bausch and Lomb glass stage micrometer slide ruled 0.1/0.01 mm. The micrometer image was centered and focused with the viewing camera. The line selector was positioned through the micrometer ruling with the marker dot superimposed over the ruling. The linear measurement unit has scale factor calibration controls, these were adjusted to provide a digital display readout of 0.000 to 2.500 mm. - 0.005 mm.

The measurement of the tetracycline fluorescent intensity was made by passing first one then the other of the unidentified and previously selected ground sections of each pair of incisors through the center point of the ultraviolet light. The intensity of each band of fluorescing tetracycline was measured individually starting from the outer enamel surface and ending on a tangent to the pulp chamber. The pulp canal was not included in the measurement to eliminate its intense autofluorescence from distorting the results (Figures 6 and 7). The maxillary incisor was measured by centering the scan line through the central groove of the labial surface. The mandibular incisor was measured by aligning the scan line perpendicular to the mid-point of the labial surface (Figure 3).

Photomicrographs for publication were taken of selected sections with a Leitz ultraviolet light microscope with BG-12, BG-38 exciter filters and a 53 insert barrier filter. A Pentax camera and microscope adapter with Hi-Speed Ektachrome (ASA 165) were used. Exposure time was between 5 and 60 seconds.

Analysis of Data

The raw data were adjusted by computer to calculate the individual intensity of each band of tetracycline in a ground section. Intensity of fluorescence equaled the peak amplitude of the fluorescence minus the peak amplitude of the electronic noise and the base of the wave form (Figure 5). The numerical unit assigned equaled 1/1000 of the total oscilloscope height; since the signal was amplified and the oscilloscope was modified, no absolute unit (volts, lumens) could be assigned these values.

The mean of 6 to 17 individual intensities from the tetracycline bands in a tooth was used as the fluorescent intensity of the tooth. This value for each tooth was then used to obtain a mean for a given group of teeth.

The difference in intensity of right and left incisor or unbleached and bleached incisor was obtained for each fluorescent band of the paired teeth and mean differences for the paired teeth and for a group of paired teeth were calculated.

Using these means, t-test comparisons for statistical significance were computed: A) between each pair of teeth in the same arch of each rabbit; B) between all teeth that were unbleached compared to all that were bleached in the entire group; and C) between all teeth of the control group, all teeth that had been bleached once and all teeth that had been bleached twice.

Observations made during a review of the Kodachrome slides taken during the clinical bleaching were then correlated with the measurement data.

RESULTS

At the time of clinical bleaching, a shade guide was used for comparison with the rabbits' incisors. The rabbits with no oxytetracycline exhibited a shade of approximately 61 on a Trubyte Biotone shade guide; those rabbits who had received oxytetracycline exhibited a color approximating shade 69, yet the teeth exhibited a yellow hue not present in this or the Sevriton shade guide. This verified that the teeth of rabbits which had received oxytetracycline were different in color from those rabbits which had not received the drug and that tetracycline staining had been induced. Kodachromes of the rabbits taken in white and ultraviolet light during the clinical bleaching (Figures 9 and 10) show that the maxillary incisors bleached clinically more successfully, as their shade became closer to shade 61 after the bleaching than the mandibular incisors. Table XIX compares the clinical Kodachromes with the depth of bleaching measured in the ground sections.

The strongest bleaching occurred in the incisal one-third in both maxillary and mandibular incisors and was approximately 250 to 350 microns into the dentin in the maxillary and 150 to 200 microns into the dentin in the mandibular incisors. An important observation is that the tetracycline fluorescence was not entirely removed by the bleaching process (Figures 11 and 12). The tetracycline fluorescence appeared microscopically to be little changed by the bleach, except that its ability to fluoresce was slightly reduced and the bands exhibited less contrast than in the unbleached specimens. Tetracycline-stained teeth which were

not bleached exhibited a gradual increase of tetracycline fluorescent intensity from the outer enamel surface to the pulp canal. This pattern of fluorescent intensity was altered in about one-half of the cases surveyed, with the last three or four bands demonstrating a subtle decrease in fluorescent intensity. A graphic demonstration of these observations is detailed in the portion of the computer print-out reproduced from Appendix II that follows:

RABBIT 12 MAXILLARY MIDDLE THIRD--TWO BLEACHES*

I-1 (unbleached)	I-2 (bleached)	I-3 (unbl.-bl.)	D-1 (depth I-1)	D-2 (depth I-2)
233	103	130	72	52**
257	94	163	104	83
260	100	160	134	110
254	126	128	160	124
260	139	121	186	162
294	153	141	210	192
310	180	130	237	222
317	197	120	260	248**
307	212	95	287	269
306	219	87	312	292
320	242	78	336	315
300	247	53	364	345
267	237	30	388	372
257	213	44	400	400

N = 14

*The computer print-out demonstrates the fluorescent intensity of unbleached (I-1) and bleached (I-2) dentin. Each line represents a band of tetracycline. I-3 is the difference (I-1 - I-2) and represents the loss of intensity per band. D-1 corresponds to I-1 and D-2 corresponds to I-2 and is the depth of the leading edge of the tetracycline band from the outer enamel surface; the distance is in microns. The unit for intensity is 1/1000 of the oscilliscope height.

**At the point of the line one can see that the greatest bleaching stopped and was between 52 to 260 microns. The intensity of tetracycline fluorescence gradually changed from the first band to the last band in column I-1 and I-2. Refer to Appendix I for additional examples.

The data in Table II through Table V demonstrate that there was no statistically significant difference between bleached and unbleached non-stained teeth. The bleaching process did not alter the inherent fluorescence of the enamel and dentin. In one case (mandibular middle third after one bleach--Table III) where there was a significant difference at the <0.05 level, the fluorescence was more intense in the bleached than in the unbleached teeth. The intensity of the unstained teeth was very low and no peaks of fluorescence could be seen on the oscilloscope above the peak of the electronic noise. To achieve a value for the fluorescence of unstained teeth, the peak value of the electronic noise was used as the peak of fluorescence of the unstained teeth.

The enamel of tetracycline-stained teeth fluoresced only slightly above the values obtained for unstained teeth, averaging about 10 units of fluorescent intensity (Tables VI to XI). This is in agreement with current knowledge that fluorescence of tetracycline is not seen to any great extent in the enamel. The difference in the intensity of tetracycline fluorescence of enamel as opposed to the fluorescence of tetracycline in dentin was highly significant. This significance is demonstrated by computing the t-test value from the mean intensities of the enamel and dentin of the unbleached teeth in the incisal one-third (Table X and Table XVI). If the t value is greater than 3.05, there is significance at the .01 level when there are 12 degrees of freedom. The computation of the t value is as follows;

	Intensity	Standard Error	Degrees of Freedon	t-test value
Enamel	5.57	2.54		
			12	8.85
Dentin	268	29.6		

Therefore, a highly significant difference is present between the degree of fluorescence of dentin and the degree of fluorescence of enamel in tetracycline teeth. Since the fluorescence of tetracycline in enamel is minimal, the presence or absence of tetracycline in enamel cannot be accurately determined by its fluorescence.

Tables VIII through XI show that there was some effect of the bleaching procedure on the tetracycline in enamel, with an average decrease in fluorescent intensity of about 2 units after one bleach and about 7 units after two bleaches. This was statistically significant in only one group of animals (Table X--maxillary middle third); in this group the bleached enamel fluoresced with a significantly lower intensity ($P < 0.05$) than the unbleached enamel.

Tables XII and XIII show that there was great variation between incisors in the same arch. In a comparison of 36 pairs of teeth, 18 showed a significant difference: in 13 cases the right incisor showed a higher intensity of fluorescence, and in five cases the left showed a higher intensity. This finding invalidated the use of tetracycline-stained incisors of individual rabbits to act as their own controls for unbleached and bleached teeth.

In comparing groups of teeth (Tables XII through XVIII), there was an insignificant statistical difference between right and left rabbit incisors which were stained with tetracycline and not bleached. There was a tendency for reduced tetracycline fluorescence, with an average lowering of about 45 units of intensity after one bleach and a statistically significant loss of tetracycline fluorescence in the maxillary incisors

($P < 0.001$) and in the incisal one-third of the mandibular incisors ($P < 0.005$) after two bleaches. Therefore, groups of teeth can be compared and there was an effect when the tetracycline-stained incisors were bleached twice.

TABLES AND FIGURES

TABLE II

Comparison of dentin and enamel fluorescence of unbleached and bleached once maxillary rabbit incisors which received no tetracycline. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means.*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Incisal Third								
Rabbit 1-section A	40.0**		45.0		-5.00			
Rabbit 1-section B	45.0		40.0		5.00			
Rabbit 1-section C	60.0		40.0		14.0			
Rabbit 1-section D	72.0		62.0		10.0			
Rabbit 4-section A	73.0		63.0		10.0			
Rabbit 4-section B	52.0		42.0		10.0			
Rabbit 4-section C	90.0		44.0		46.0			
Mean	61.7	6.69	48.7	3.62	12.8	5.98	1.71	NS
Middle Third								
Rabbit 1-section A	62.0		90.0		-28.0			
Rabbit 1-section B	85.0		68.0		17.0			
Rabbit 1-section C	72.0		71.0		1.00			
Rabbit 1-section D	72.0		72.0		0.00			
Rabbit 4-section A	78.0		41.0		37.0			
Rabbit 4-section B	87.0		81.0		6.00			
Rabbit 4-section C	90.0		79.0		11.0			
Rabbit 4-section D	54.0		78.0		-24.0			
Mean	75.0	4.44	72.5	5.11	2.50	7.47	.361	NS

*Obtained by means of a t-test. When difference is not significant the letter NS are used instead of P,
**Each unit represents 1/1000 of total height of oscilloscope.

TABLE II (continued)

Comparison of dentin and enamel fluorescnece of unbleached and bleached once maxillary rabbit incisors which received no tetracycline. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means.*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Gingival Third								
Rabbit 1-section A	45.0**		90.0		-45.0			
Rabbit 1-section B	65.0		85.0		-20.0			
Rabbit 1-section C	79.0		78.0		1.00			
Rabbit 1-section D	98.0		65.0		33.0			
Rabbit 4-section A	79.0		76.0		3.00			
Rabbit 4-section B	75.0		86.0		-11.0			
Rabbit 4-section C	87.0		93.0		-6.00			
Rabbit 4-section D	64.0		86.0		-22.0			
Mean	74.0	5.70	82.4	3.18	-8.38	8.02	1.29	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of total height of the oscilloscope.

TABLE III

Comparison of dentin and enamel fluorescence of unbleached and bleached once mandibular rabbit incisors which received no tetracycline. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Incisal Third								
Rabbit 1-section A	82.0**		87.0		-5.00			
Rabbit 1-section B	85.0		77.0		8.00			
Rabbit 1-section C	98.0		85.0		13.0			
Rabbit 4-section A	53.0		97.0		-44.0			
Rabbit 4-section B	64.0		78.0		-14.0			
Rabbit 4-section C	74.0		72.0		2.00			
Mean	76.0	6.53	82.7	3.64	-5.00	8.65	.896	NS
Middle Third								
Rabbit 1-section A	71.0		85.0		-14.0			
Rabbit 1-section B	86.0		98.0		-12.0			
Rabbit 1-section C	80.0		78.0		2.00			
Rabbit 4-section A	70.0		88.0		-18.0			
Rabbit 4-section B	57.0		77.0		-20.0			
Rabbit 4-section C	63.0		88.0		-25.0			
Mean	71.2	4.35	85.7	3.15	-14.5	3.79	2.70	<.05
Gingival Third								
Rabbit 1-section A	89.0		92.0		-3.00			
Rabbit 1-section B	105		77.0		28.0			
Rabbit 1-section C	76.0		83.0		-7.00			
Rabbit 4-section A	80.0		84.0		-4.00			
Rabbit 4-section B	96.0		82.0		14.0			
Rabbit 4-section C	77.0		83.0		-6.00			
Mean	87.2	4.76	83.5	1.98	3.67	5.80	.715	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE IV

Comparison of dentin and enamel fluorescence of unbleached and bleached twice maxillary rabbit incisors which received no tetracycline. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Incisal Third								
Rabbit 3-section A	98.0**		90.0		8.00			
Rabbit 3-section B	105		103		2.00			
Rabbit 3-section C	102		113		-11.0			
Rabbit 21-section A	107		110		-3.00			
Rabbit 21-section B	105		125		-20.0			
Rabbit 21-section C	111		118		-7.00			
Rabbit 21-section D	119		111		8.00			
Mean	107	2.55	110	4.22	-3.28	3.89	.608	NS
Middle Third								
Rabbit 3-section A	109		114		-5.00			
Rabbit 3-section B	80.0		115		-35.0			
Rabbit 3-section C	101		110		-9.00			
Rabbit 3-section D	98.0		106		-8.00			
Rabbit 21-section A	111		117		-6.00			
Rabbit 21-section B	111		102		9.00			
Rabbit 21-section C	105		100		5.00			
Rabbit 21-section D	107		122		-15.0			
Mean	103	3.64	111	2.70	-8.00	4.72	1.76	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of total height of the oscilloscope.

TABLE IV (continued)

Comparison of dentin and enamel fluorescence of unbleached and bleached twice maxillary rabbit incisors which received no tetracycline. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Gingival Third								
Rabbit 3-section A	94.0**		86.0		8.00			
Rabbit 3-section B	91.0		102		-9.00			
Rabbit 3-section C	88.0		108		-20.0			
Rabbit 21-section A	87.0		92.0		-5.00			
Rabbit 21-section B	112		137		-25.0			
Rabbit 21-section C	119		123		-4.00			
Rabbit 21-section D	120		119		1.00			
Mean	102	5.60	110	6.81	-7.71	4.35	.907	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of total height of the oscilloscope.

TABLE V

Comparison of dentin and enamel fluorescence of unbleached and bleached twice mandibular rabbit incisors which received no tetracycline. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Incisal Third								
Rabbit 3-section A	118**		88.0		30.0			
Rabbit 3-section B	105		97.0		8.00			
Rabbit 21-section A	88.0		107		-19.0			
Rabbit 21-section B	86.0		95.0		-9.00			
Rabbit 21-section C	93.0		88.0		5.00			
Mean	98.0	5.99	95.0	3.51	3.00	8.32	.432	NS
Middle Third								
Rabbit 3-section A	100		97.0		3.00			
Rabbit 3-section B	98.0		98.0		0.00			
Rabbit 3-section C	105		105		0.00			
Rabbit 21-section A	101		98.0		3.00			
Rabbit 21-section B	105		97.0		8.00			
Rabbit 21-section C	100		97.0		3.00			
Mean	102	1.39	98.5	1.37	2.83	1.40	1.54	NS
Gingival Third								
Rabbit 3-section A	101		98.0		3.00			
Rabbit 3-section B	112		96.0		16.0			
Rabbit 3-section C	107		110		-3.00			
Rabbit 3-section D	102		113		-11.0			
Rabbit 21-section A	88.0		114		-26.0			
Rabbit 21-section B	91.0		109		-18.0			
Rabbit 21-section C	104		92.0		12.0			
Rabbit 21-section D	100		79.0		21.0			
Mean	101	2.79	101	4.34	-0.75	5.94	0.00	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE VI

Comparison of enamel tetracycline fluorescent intensity of right and left maxillary rabbit incisors. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Right	SE	Left	SE	Difference	SE	t	P
Incisal Third								
Rabbit 10A**	28.0***		27.0		1.00			
Rabbit 10B	47.0		34.0		13.0			
Rabbit 13A	27.0		21.0		6.00			
Rabbit 13B	4.00		10.0		-6.00			
Rabbit 15A	17.0		0.00		17.0			
Rabbit 15B	10.0		3.00		7.00			
Mean	22.2	6.27	15.8	5.57	6.33	3.36	.763	NS
Middle Third								
Rabbit 10A	-10.0		27.0		-37.0			
Rabbit 10B	25.0		68.0		-43.0			
Rabbit 13A	26.0		11.0		15.0			
Rabbit 13B	20.0		19.0		1.00			
Rabbit 15A	3.00		4.00		-1.00			
Rabbit 15B	-9.00		35.0		-44.0			
Mean	9.17	6.80	27.3	9.30	-18.2	10.6	1.57	NS
Gingival Third								
Rabbit 10A	48.0		90.0		42.0			
Rabbit 10B	56.0		46.0		-10.0			
Rabbit 13A	37.0		20.0		17.0			
Rabbit 13B	17.0		9.00		8.00			
Rabbit 15A	43.0		14.0		29.0			
Rabbit 15B	1.00		22.0		-21.0			
Mean	33.7	8.46	33.5	12.4	10.8	9.65	.013	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**A and B represent different sections from the same animal in that third.
***Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE VII

Comparison of enamel tetracycline fluorescent intensity of right and left mandibular rabbit incisors. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Right	SE	Left	SE	Difference		t	P
Incisal Third								
Rabbit 10A**	4.00***		-12.0		16.0			
Rabbit 10B	-9.00		0.00		-9.00			
Rabbit 13A	15.0		7.00		8.00			
Rabbit 13B	21.0		1.00		20.0			
Rabbit 15A	11.0		-3.00		14.0			
Rabbit 15B	11.0		4.00		7.00			
Mean	8.83	4.23	-0.50	2.69	9.33	4.18	1.86	NS
Middle Third								
Rabbit 10A	8.00		2.00		6.00			
Rabbit 10B	11.0		3.00		8.00			
Rabbit 13A	6.00		17.0		-11.0			
Rabbit 13B	24.0		19.0		5.00			
Rabbit 15A	13.0		0.00		13.0			
Rabbit 15B	17.0		2.00		15.0			
Mean	13.2	2.68	7.17	3.46	6.00	3.76	1.38	NS
Gingival Third								
Rabbit 10A	-8.00		-2.00		-6.00			
Rabbit 10B	10.0		6.00		4.00			
Rabbit 13A	9.00		22.0		-13.0			
Rabbit 13B	18.0		8.00		10.0			
Rabbit 15A	5.00		0.00		5.00			
Rabbit 15B	-13.0		18.0		-31.0			
Mean	3.50	4.79	8.67	3.92	-5.17	6.18	.835	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**A and B represent different sections from the same animal in that third.
***Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE VIII

Comparison of enamel tetracycline fluorescent intensity of unbleached and bleached once maxillary rabbit incisors. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Incisal Third								
Rabbit 6	10.0**		-15.0		25.0			
Rabbit 8	17.0		11.0		6.00			
Rabbit 9	2.00		8.00		-6.00			
Rabbit 11	9.00		11.0		-2.00			
Mean	9.50	3.70	3.75	6.29	5.60	6.28	.788	NS
Middle Third								
Rabbit 6	5.00		0.00		5.00			
Rabbit 8	40.0		5.00		35.0			
Rabbit 9	-9.00		18.0		-27.0			
Rabbit 11	8.00		16.0		-8.00			
Mean	11.0	10.4	9.75	4.20	1.25	12.8	.111	NS
Gingival Third								
Rabbit 6	15.0		1.00		14.0			
Rabbit 8	16.0		8.00		8.00			
Rabbit 9	16.0		27.0		-11.0			
Rabbit 11	34.0		14.0		20.0			
Mean	20.2	4.59	12.5	5.52	7.75	6.71	1.07	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of total height of the oscilloscope.

TABLE IX

Comparison of enamel tetracycline fluorescent intensity of unbleached and bleached once mandibular rabbit incisors. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Incisal Third								
Rabbit 6	31.0**		-5.00		36.0			
Rabbit 8	3.00		41.0		-38.0			
Rabbit 9	-5.00		8.00		-13.0			
Rabbit 11	-1.00		-11.0		10.0			
Mean	7.00	8.16	8.25	11.6	-1.25	15.8	.088	NS
Middle Third								
Rabbit 6	0.00		0.00		0.00			
Rabbit 8	5.00		0.00		5.00			
Rabbit 9	8.00		5.00		3.00			
Rabbit 11	7.00		24.0		-17.0			
Mean	5.00	1.78	7.25	5.70	-2.25	5.02	.377	NS
Gingival Third								
Rabbit 6	-5.00		8.00		-13.0			
Rabbit 8	5.00		12.0		-7.00			
Rabbit 9	13.0		-10.0		23.0			
Rabbit 11	35.0		36.0		-1.00			
Mean	12.0	8.50	11.5	9.46	0.50	7.89	.039	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of total height of the oscilloscope.

TABLE X

Comparison of enamel tetracycline fluorescent intensity of unbleached and bleached twice maxillary rabbit incisors. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Incisal Third								
Rabbit 7	2.00**		2.00		0.00			
Rabbit 12	10.0		0.00		10.0			
Rabbit 16	10.0		9.00		1.00			
Rabbit 17	12.0		10.0		2.00			
Rabbit 18	-6.00		-11.0		5.00			
Rabbit 19	2.00		-14.0		16.0			
Rabbit 20	9.00		-6.00		15.0			
Mean	5.57	2.54	-1.43	3.53	7.00	2.46	1.63	NS
Middle Third								
Rabbit 7	5.00		7.00		-2.00			
Rabbit 12	12.0		-3.00		15.0			
Rabbit 16	18.0		6.00		12.0			
Rabbit 17	19.0		13.0		6.00			
Rabbit 18	19.0		4.00		15.0			
Rabbit 19	15.0		-5.00		20.0			
Rabbit 20	13.0		-5.00		18.0			
Mean	14.4	1.90	2.43	2.62	12.0	2.89	2.41	<.05

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each number represents 1/1000 of the total height of the oscilloscope.

TABLE X (continued)

Comparison of enamel tetracycline fluorescent intensity of unbleached and bleached twice maxillary rabbit incisors. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Gingival Third								
Rabbit 7	17.0**		-16.0		33.0			
Rabbit 12	19.0		10.0		9.00			
Rabbit 16	15.0		4.00		11.0			
Rabbit 17	38.0		22.0		16.0			
Rabbit 18	9.00		-16.0		25.0			
Rabbit 19	10.0		9.00		1.00			
Rabbit 20	7.00		7.00		0.00			
Mean	16.4	3.96	2.86	5.31	13.6	4.59	2.04	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each number represents 1/1000 of the total height of the oscilloscope.

TABLE XI

Comparison of enamel tetracycline fluorescent intensity of unbleached and bleached twice mandibular rabbit incisors. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Incisal Third								
Rabbit 7	1.00**		10.0		-9.00			
Rabbit 12	9.00		3.00		6.00			
Rabbit 14	62.0		-5.00		67.0			
Rabbit 16	2.00		-6.00		8.00			
Rabbit 17	21.0		-3.00		24.0			
Rabbit 18	6.00		0.00		6.00			
Rabbit 19	-2.00		-7.00		5.00			
Rabbit 20	-6.00		-16.0		10.0			
Mean	11.6	7.75	-3.00	2.71	14.6	8.12	1.77	NS
Middle Third								
Rabbit 7	8.00		2.00		6.00			
Rabbit 12	6.00		13.0		-7.00			
Rabbit 14	1.00		5.00		-4.00			
Rabbit 16	15.0		8.00		7.00			
Rabbit 17	33.0		10.0		23.0			
Rabbit 18	3.00		0.00		3.00			
Rabbit 19	20.0		0.00		20.0			
Rabbit 20	-2.00		-10.0		8.00			
Mean	10.5	4.11	3.50	2.55	7.00	3.68	1.44	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE XI (continued)

Comparison of enamel tetracycline fluorescent intensity of unbleached and bleached twice mandibular rabbit incisors. The incisal, middle and gingival thirds of the teeth were measured.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached	SE	Bleached	SE	Difference	SE	t	P
Gingival Third								
Rabbit 7	6.00**		22.0		-16.0			
Rabbit 12	13.0		-5.00		18.0			
Rabbit 14	28.0		22.0		6.00			
Rabbit 16	48.0		14.0		34.0			
Rabbit 17	30.0		15.0		15.0			
Rabbit 18	11.0		5.00		6.00			
Rabbit 19	-3.00		15.0		-18.0			
Rabbit 20	-11.0		-2.00		-9.00			
Mean	15.2	6.79	10.8	3.64	4.50	6.38	.571	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE XII

Comparison of dentin tetracycline fluorescent intensity of right and left maxillary rabbit incisors. From six to seventeen bands were measured in the incisal, middle and gingival thirds of the teeth.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Right		Left		Difference			
	Mean	SE	Mean	SE	Mean		t	P
Incisal Third								
Rabbit 10A**	187***	11.6	196	3.24	-8.89	12.7	0.75	NS
Rabbit 10B	223	3.28	209	2.71	13.8	4.78	3.29	<0.005
Rabbit 13A	270	6.08	280	7.42	-9.63	5.21	1.04	NS
Rabbit 13B	287	8.89	257	11.4	29.9	6.93	2.08	<0.05
Rabbit 15A	213	11.5	235	15.4	-21.6	19.2	1.14	NS
Rabbit 15B	195	8.80	191	7.09	4.43	9.26	0.35	NS
Mean	229	16.6	228	14.5	1.34	7.60	.045	NS
Middle Third								
Rabbit 10A	203	5.80	275	10.6	-72.6	6.31	5.96	<0.001
Rabbit 10B	308	8.39	296	6.35	12.5	5.33	1.44	NS
Rabbit 13A	321	12.1	270	6.84	51.1	8.98	3.67	<0.001
Rabbit 13B	307	6.64	259	9.18	47.7	7.51	4.24	<0.001
Rabbit 15A	272	5.14	225	7.50	47.3	4.65	5.17	<0.001
Rabbit 15B	245	9.06	248	4.45	-3.00	9.46	0.30	NS
Mean	276	18.5	262	9.93	13.8	19.5	.667	NS
Gingival Third								
Rabbit 10A	183	6.20	222	10.6	-39.5	5.93	3.18	<0.005
Rabbit 10B	254	9.46	228	10.7	-26.4	1.82	1.82	NS
Rabbit 13A	240	9.90	204	7.30	35.7	5.87	2.93	<0.01
Rabbit 13B	232	8.90	184	13.5	48.0	2.97	2.97	<0.01
Rabbit 15A	218	4.21	195	3.84	22.5	3.04	4.04	<0.001
Rabbit 15B	229	7.40	193	3.26	35.8	8.17	4.45	<0.001
Mean	226	9.89	204	7.07	12.7	12.7	1.80	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**A and B represent different sections from the same animal in that third.
***Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE XIII

Comparison of dentin tetracycline fluorescent intensity of right and left mandibular rabbit incisors. From six to seventeen bands were measured in the incisal, middle and gingival thirds of the teeth.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Right		Left		Difference			
	Mean	SE	Mean	SE	Mean	SE	t	P
Incisal Third								
Rabbit 10A**	217***	3.87	220	4.54	-3.18	4.42	0.50	NS
Rabbit 10B	210	5.62	217	6.50	-7.34	6.63	0.81	NS
Rabbit 13A	265	18.7	260	12.7	4.69	13.6	0.22	NS
Rabbit 13B	267	13.8	222	14.1	44.8	6.25	2.28	<0.05
Rabbit 15A	191	6.55	215	5.96	-24.5	5.15	2.71	<0.02
Rabbit 15B	200	10.2	191	5.38	8.28	10.3	0.78	NS
Mean	225	13.4	221	9.08	3.79	9.44	.249	NS
Middle Third								
Rabbit 10A	231	6.42	233	9.52	-1.80	5.46	0.17	NS
Rabbit 10B	243	8.13	224	6.64	19.2	4.92	1.81	NS
Rabbit 13A	325	13.2	273	12.0	52.2	7.49	2.91	<0.01
Rabbit 13B	298	14.3	252	6.47	45.8	11.3	2.93	<0.01
Rabbit 15A	234	10.3	240	9.42	-5.81	6.07	0.43	NS
Rabbit 15B	198	6.83	199	6.04	-1.13	5.51	0.11	NS
Mean	255	19.3	237	10.3	18.1	10.4	.823	NS
Gingival Third								
Rabbit 10A	211	9.61	189	10.6	21.2	4.16	1.53	NS
Rabbit 10B	243	7.84	212	7.22	30.9	5.93	2.91	<0.01
Rabbit 13A	279	19.6	294	18.7	-15.2	6.27	0.55	NS
Rabbit 13B	285	14.7	251	21.3	34.0	10.1	1.31	NS
Rabbit 15A	230	9.19	262	10.5	-31.8	3.84	2.29	<0.05
Rabbit 15B	297	7.16	228	12.8	68.5	8.90	4.70	<0.001
Mean	258	14.0	239	15.3	17.9	14.8	.916	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**A and B represent different ground sections from the same animal in that third.
***Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE XIV

Comparison of dentin tetracycline fluorescent intensity of unbleached and bleached once maxillary rabbit incisors. From six to seventeen bands were measured in incisal, middle and gingival thirds of the teeth.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached		Bleached		Difference			
	Mean	SE	Mean	SE	Mean	SE	t	P
Incisal Third								
Rabbit 6	153**	8.53	145	4.19	7.33	8.20	0.84	NS
Rabbit 8	356	8.54	252	7.14	104	6.58	9.34	<0.001
Rabbit 9	297	11.6	275	10.0	22.6	5.74	1.44	NS
Rabbit 11	343	9.58	309	16.2	33.4	17.5	1.80	NS
Mean	287	46.5	245	35.4	41.8	21.4	.718	NS
Middle Third								
Rabbit 6	174	4.22	140	4.87	33.9	5.89	5.27	<0.01
Rabbit 8	374	7.08	272	13.1	102	17.5	6.84	<0.01
Rabbit 9	302	11.8	288	7.58	13.4	11.4	1.00	NS
Rabbit 11	370	14.4	312	21.3	58.1	27.0	2.26	<0.05
Mean	305	46.7	253	38.6	51.8	19.0	.858	NS
Gingival Third								
Rabbit 6	216	3.86	205	4.18	11.6	2.68	1.93	NS
Rabbit 8	366	10.6	291	9.02	74.4	7.07	5.39	<0.001
Rabbit 9	173	4.52	152	5.76	20.9	5.13	2.87	<0.01
Rabbit 11	415	11.3	322	15.2	93.8	5.73	4.91	<0.001
Mean	292	58.1	242	39.0	50.2	20.0	.714	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE XV

Comparison of dentin tetracycline fluorescent intensity of unbleached and bleached once mandibular rabbit incisors. From six to seventeen bands were measured in incisal, middle and gingival thirds of the teeth.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached		Bleached		Difference			
	Mean	SE	Mean	SE	Mean	SE	t	P
Incisal Third								
Rabbit 6	151**	6.44	137	6.94	14.0	2.48	1.48	NS
Rabbit 8	115	5.83	69.4	3.71	46.0	4.69	6.60	<0.001
Rabbit 9	265	13.7	277	19.6	-11.7	9.45	0.50	NS
Rabbit 11	276	10.1	193	8.14	82.9	7.61	6.40	<0.001
Mean	202	40.4	169	44.0	32.8	20.4	.552	NS
Middle Third								
Rabbit 6	100	6.94	118	8.79	-17.9	5.23	1.61	NS
Rabbit 8	150	12.2	91.3	4.97	59.1	9.30	4.46	<0.001
Rabbit 9	331	15.2	294	23.6	36.4	11.5	1.32	NS
Rabbit 11	358	16.1	269	13.2	88.3	6.80	4.27	<0.001
Mean	235	64.4	193	51.6	41.5	22.5	.509	NS
Gingival Third								
Rabbit 6	129	7.34	86.5	7.45	42.8	4.19	4.06	<0.001
Rabbit 8	364	24.4	264	12.0	99.6	13.5	3.68	<0.005
Rabbit 9	324	12.1	239	26.7	85.2	16.9	2.90	<0.01
Rabbit 11	317	12.5	366	15.8	-48.9	7.17	2.43	<0.05
Mean	284	52.5	239	57.7	44.7	33.4	.597	NS

*Obtained by means of a t-test. When differnece is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE XVI

Comparison of dentin tetracycline fluorescent intensity of unbleached and bleached twice maxillary rabbit incisors. From six to seventeen bands were measured in incisal, middle and gingival thirds of the teeth.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached		Bleached		Difference			
	Mean	SE	Mean	SE	Mean	SE	t	P
Incisal Third								
Rabbit 7	332**	8.00	149	3.12	183	5.50	21.3	<0.001
Rabbit 12	246	6.80	149	10.3	97.8	5.16	7.86	<0.001
Rabbit 16	319	16.0	212	13.3	108	7.47	5.14	<0.001
Rabbit 17	380	18.0	329	14.5	51.5	24.7	2.21	<0.05
Rabbit 18	239	17.9	146	14.7	93.3	16.4	4.01	<0.001
Rabbit 19	204	10.4	148	14.1	56.4	9.06	3.20	<0.005
Rabbit 20	158	10.6	22.7	5.21	135	7.49	11.4	<0.001
Mean	268	29.6	165	34.7	103	17.2	2.26	0.05
Middle Third								
Rabbit 7	366	9.95	260	22.7	106	25.5	4.28	<0.001
Rabbit 12	282	7.68	176	14.9	106	11.3	6.32	<0.001
Rabbit 16	382	11.2	274	8.15	108	4.77	7.80	<0.001
Rabbit 17	435	14.0	305	13.2	130	7.30	6.76	<0.001
Rabbit 18	252	15.1	149	14.1	103	12.4	4.98	<0.001
Rabbit 19	237	10.3	184	9.94	52.9	13.2	3.70	<0.001
Rabbit 20	197	6.96	58.3	9.20	139	7.34	12.0	<0.001
Mean	307	33.1	201	32.2	106	10.3	2.30	0.05

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of total height of the oscilloscope.

TABLE XVI (continued)

Comparison of dentin tetracycline fluorescent intensity of unbleached and bleached twice maxillary rabbit incisors. From six to seventeen bands were measured in incisal, middle and gingival thirds of the teeth.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached		Bleached		Difference			
	Mean	SE	Mean	SE	Mean	SE	t	P
Gingival Third								
Rabbit 7	344	5.89	242	14.6	103	13.7	6.48	<0.001
Rabbit 12	327	10.8	215	14.1	113	12.9	6.30	<0.001
Rabbit 16	384	10.2	289	9.73	95.2	4.98	6.74	<0.001
Rabbit 17	409	17.7	328	14.7	81.3	7.98	3.52	<0.005
Rabbit 18	298	17.1	178	12.1	121	19.1	5.73	<0.001
Rabbit 19	284	11.4	242	9.94	42.0	12.8	2.78	<0.01
Rabbit 20	160	7.40	79.8	8.19	80.6	9.97	7.26	<0.001
Mean	315	30.8	225	30.3	90.9	9.94	2.08	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of total height of the oscilloscope.

TABLE XVII

Comparison of dentin tetracycline fluorescent intensity of unbleached and bleached twice mandibular rabbit incisors. From six to seventeen bands were measured in incisal, middle and gingival thirds of the teeth.

Rabbit Number	Fluorescent Intensity						Statistical Significance of Difference of Means*	
	Unbleached		Bleached		Difference			
	Mean	SE	Mean	SE	Mean	SE	t	P
Incisal Third								
Rabbit 7	308**	9.59	173	13.0	135	10.2	8.36	<0.001
Rabbit 12	299	24.1	175	15.9	124	10.1	4.29	<0.001
Rabbit 14	417	11.8	177	10.6	240	5.37	15.1	<0.001
Rabbit 16	286	8.04	151	24.4	134	19.1	5.25	<0.001
Rabbit 17	324	11.0	243	20.2	81.1	14.2	3.52	<0.005
Rabbit 18	74.4	3.98	37.1	3.98	37.4	5.41	6.63	<0.001
Rabbit 19	264	19.5	100	11.9	163	13.0	7.18	<0.001
Rabbit 20	82.5	5.72	84.8	5.92	-2.31	4.45	0.28	NS
Mean	257	42.0	143	23.0	114	26.6	2.38	<0.05
Middle Third								
Rabbit 7	279	8.75	287	16.7	-8.38	10.4	0.42	NS
Rabbit 12	351	25.7	303	22.0	48.3	6.70	1.41	NS
Rabbit 14	381	11.4	196	13.8	184	6.01	10.3	<0.001
Rabbit 16	240	10.1	256	21.2	-16.1	14.2	0.68	NS
Rabbit 17	402	14.1	239	15.6	162	14.2	7.75	<0.001
Rabbit 18	99.1	9.00	99.2	7.78	-0.10	2.67	.008	NS
Rabbit 19	291	9.54	109	12.2	182	8.20	11.8	<0.001
Rabbit 20	96.9	5.19	83.1	7.52	13.8	4.96	1.42	NS
Mean	268	41.6	196	31.3	70.7	31.6	1.38	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE XVII (continued)

Comparison of dentin tetracycline fluorescent intensity of unbleached and bleached twice mandibular rabbit incisors. From six to seventeen bands were measured in incisal, middle and gingival thirds of the teeth.

Rabbit Number	Fluorescent Intensities						Statistical Significance of Difference of Means*	
	Unbleached		Bleached		Difference			
	Mean	SE	Mean	SE	Mean	SE	t	P
Gingival Third								
Rabbit 7	313**	15.3	299	12.6	14.2	6.61	0.70	NS
Rabbit 12	344	18.8	267	29.0	76.9	15.0	2.23	<0.05
Rabbit 14	361	14.1	330	14.7	31.4	11.1	1.52	NS
Rabbit 16	338	19.0	301	24.2	37.2	9.81	1.20	NS
Rabbit 17	393	15.9	332	20.2	60.8	11.9	2.37	<0.05
Rabbit 18	232	19.1	282	17.5	-49.7	16.8	1.93	NS
Rabbit 19	190	8.53	168	20.9	21.1	20.8	0.97	NS
Rabbit 20	98.6	9.39	138	7.61	-39.3	3.64	3.26	<0.005
Mean	284	35.6	265	25.7	19.0	15.7	.438	NS

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE XVIII

Comparison of the group mean difference of tetracycline fluorescent intensity in dentin in groups of unbleached control teeth with groups of teeth bleached once or twice. The groups of teeth were divided into maxillary incisal, middle and gingival thirds and mandibular incisal, middle, and gingival thirds.

Group	Mean Difference in Fluorescent Intensity	Standard Error	t(versus Control)	Statistical Significance* P
Maxillary Incisal Third				
1 Bleach	41.8**	21.4	1.78	NS
2 Bleaches	103	17.2	5.41	<0.001
Control	1.34	7.60		
Maxillary Middle Third				
1 Bleach	51.8	19.0	1.40	NS
2 Bleaches	106	10.3	4.18	<0.001
Control	13.8	19.5		
Maxillary Gingival Third				
1 Bleach	50.2	20.0	1.58	NS
2 Bleaches	90.9	9.94	4.85	<0.001
Control	12.7	12.7		
Mandibular Incisal Third				
1 Bleach	32.8	20.4	1.29	NS
2 Bleaches	114	26.6	3.90	<0.005
Control	3.79	9.44		
Mandibular Middle Third				
1 Bleach	41.5	22.5	.944	NS
2 Bleaches	70.7	31.6	.703	NS
Control	18.1	10.4		

*Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE XVIII (continued)

Comparison of the group mean difference of tetracycline fluorescent intensity in dentin in groups of unbleached control teeth with groups of teeth bleached once or twice. The groups of teeth were divided into maxillary incisal, middle and gingival thirds and mandibular incisal, middle and gingival thirds.

Group	Mean Difference in Fluorescent Intensity	Standard Error	t(versus Control)	Statistical Significance* P
Mandibular Gingival Third				
1 Bleach	44.7**	33.4	.753	NS
2 Bleaches	19.0	15.7	.051	NS
Control	17.9	14.8		

* Obtained by means of a t-test. When difference is not significant the letters NS are used instead of P.
**Each unit represents 1/1000 of the total height of the oscilloscope.

TABLE XIX

Comparison of clinical Kodachromes taken in white and ultraviolet light with depth of strongest bleaching in the ground sections from those rabbits which were bleached.

Rabbit Number	Jaw	Pre-operative Color in White Light	Number of Treatments	Post-operative Color in White Light	Post-operative Color in Ultraviolet Light	Depth of Greatest Loss in Fluorescent Intensity Observed in Ground Sections: Incisal	Middle	Gingival
1*	Maxillae	gray-white	1 bleach	slightly whiter	blue	no effect	no effect	no effect
1*	Mandible	gray-white	1 bleach	slightly whiter	blue	no effect	no effect	no effect
4*	Maxillae	white	1 bleach	no change	blue	no effect	no effect	no effect
4*	Mandible	white	1 bleach	no change	blue	no effect	no effect	no effect
3*	Maxillae	white	2 bleach	no change	blue	no effect	no effect	no effect
3*	Mandible	white	2 bleach	no change	blue	no effect	no effect	no effect
21*	Maxillae	gray-white	2 bleach	slightly whiter	blue	no effect	no effect	no effect
21*	Mandible	gray-white	2 bleach	slightly whiter	blue	no effect	no effect	no effect
6	Maxillae	pale-yellow	1 bleach	slightly whiter	yellow, in incisal 1/3 slight loss of yellow	no effect	no effect	no effect
6	Mandible	pale-yellow	1 bleach	pale yellow	yellow no change	no effect	no effect	no effect
8	Maxillae	yellow-brown	1 bleach	slight whitening	yellow in incisal 1/3 turned to light blue	bleaching to 270-429 microns	even bleaching 360-432 microns	bleaching to 381 microns

*Received no oxytetracycline

TABLE XIX (continued)

Comparison of clinical Kodachromes taken in white and ultraviolet light with depth of strongest bleaching in the ground sections from those rabbits which were bleached.

Rabbit Number	Jaw	Pre-operative Color in White Light	Number of Treatments	Post-operative Color in White Light	Post-operative Color in Ultraviolet Light	Depth of Greatest Loss in Fluorescent Intensity Observed in Ground Sections: Incisal	Middle	Gingival
8	Mandible	yellow-brown	1 bleach	incisal 1/3 slight lightening to pale-yellow	incisal 1/3 slight decrease in yellow	bleached to pulp strongest at 274 microns	bleached to pulp strongest at 232 microns	from 278 to 466 microns strong bleaching
9	Maxillae	pale-yellow	1 bleach	slight lightening of yellow	very slight change in yellow	no effect	no effect	no effect
9	Mandible	pale-yellow	1 bleach	no change	yellow no change	no effect	weak bleach to 82 micron	bleach to to 142 microns
11	Maxillae	pale-yellow	1 bleach	slight lightening of incisal, middle 1/3	yellow no change	bleached 89 to 153 microns	bleached to 186 microns weaker to pulp canal	bleached to 226 microns weaker to pulp canal
11	Mandible	pale-yellow	1 bleach	slight lightening of incisal 1/3	yellow no change	no change	bleached to 255 microns weaker to 383 microns	no effect
7	Maxillae	pale-yellow	2 bleach	whole tooth whiter	incisal 1/3 turned from yellow to pale blue	bleached to 268 - 412 microns	bleached to 292 microns	strong bleach to 282, less to 446 microns

TABLE XIX (continued)

Comparison of clinical Kodachromes taken in white and ultraviolet light with depth of strongest bleaching in the ground sections from those rabbits which were bleached.

Rabbit Number	Jaw	Pre-operative Color in White Light	Number of Treatments	Post-operative Color in White Light	Post-operative Color in Ultraviolet Light	Depth of Greatest Loss in Fluorescent Intensity Observed in Ground Sections: Incisal	Middle	Gingival
7	Mandible	pale-yellow	2 bleach	incisal ½ whiter	incisal 1/3 turned from yellow to pale blue	strong to 268 less to 404 microns	bleached to 102 microns	no effect
12	Maxillae	yellow-brown	2 bleach	incisal ½ much whiter	incisal ½ turned from yellow to pale blue	strong to 303 and less to 426 microns	strong to 248 less to 400 microns	strong to 307 less to 396 microns
12	Mandible	yellow-brown	2 bleach	incisal ½ much whiter	yellow is less in incisal 1/3	weak bleach to 86 then strong from 116 to 450 microns	strong to 94 microns	strong to 118 microns
14	Maxillae--tooth fractured by animal on cage no measurements taken.							
14	Mandible	yellow	2 bleach	incisal ½ much whiter	incisal ½ turned from yellow to pale blue	very strong to 568 microns	strong to 166 microns less to 506 microns	no effect till 303 to 447 microns
16	Maxillae	pale-yellow	2 bleach	entire tooth whiter	incisal ½ turned from yellow to pale blue	strong to 234-398 microns	strong to 362 less to 413 microns	strong to 251 less to 456 microns
16	Mandible	pale-yellow	2 bleach	entire tooth whiter	incisal 1/3 less yellow	strong to 142 microns	no effect	strong to 133 microns

TABLE XIX (continued)

Comparison of clinical Kodachromes taken in white and ultraviolet light with depth of strongest bleaching in the ground sections from those rabbits which were bleached.

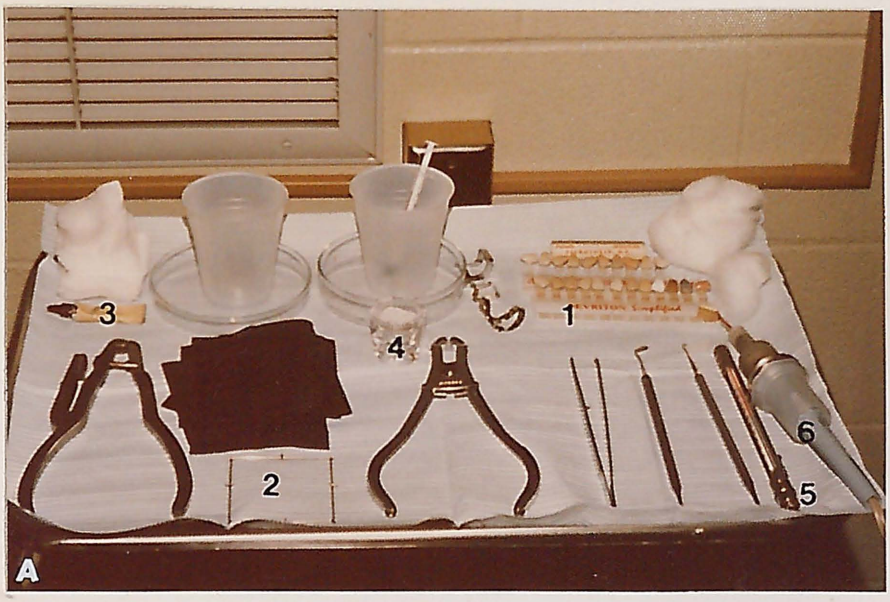
Rabbit Number	Jaw	Pre-operative Color in White Light	Number of Treatments	Post-operative Color in White Light	Post-operative Color in Ultraviolet Light	Depth of Greatest Loss in Fluorescent Intensity Observed in Ground Sections: Incisal	Middle	Gingival
17	Maxillae	gray-yellow	2 bleach	incisal ½ much whiter	less yellow incisal ½	strong to 284 less to 394 microns	strong to 378 microns	strong to 388 microns
17	Mandible	gray-yellow	2 bleach	incisal 1/3 whiter	incisal 1/3 less yellow	strong to 162 microns	strong to 140 microns	strong to 118 microns
18	Maxillae	pale-yellow	2 bleach	entire tooth much whiter	incisal ½ turned from yellow to pale blue	strong to 339 microns	strong to 260 microns	strong to 400 microns
18	Mandible	pale-yellow	2 bleach	slight whitening incisal 1/3	incisal 1/3 no effect yellow	slight to 150 microns	no effect	no effect
19	Maxillae	pale-yellow	2 bleach	incisal 1/3 very white	incisal 1/3 pale blue rest yellow	strong to 47 less to 403 microns	strong to 168	inconsistent bleach little effect.
19	Mandible	pale-yellow	2 bleach	incisal 1/3 much whiter	incisal 1/3 pale blue rest yellow	strongest to 254 less to 430 microns	strong to 161, less to 424 microns	strong only to 62 microns
20	Maxillae	yellow-brown	2 bleach	entire tooth whiter	entire tooth is a lighter yellow	strong to 452 microns	strong to 448 microns	weak bleach to 230 then strong 254 to 392 microns
20	Mandible	yellow-brown	2 bleach	slight whitening in incisal 1/3	no effect same yellow	no effect	no effect	no effect

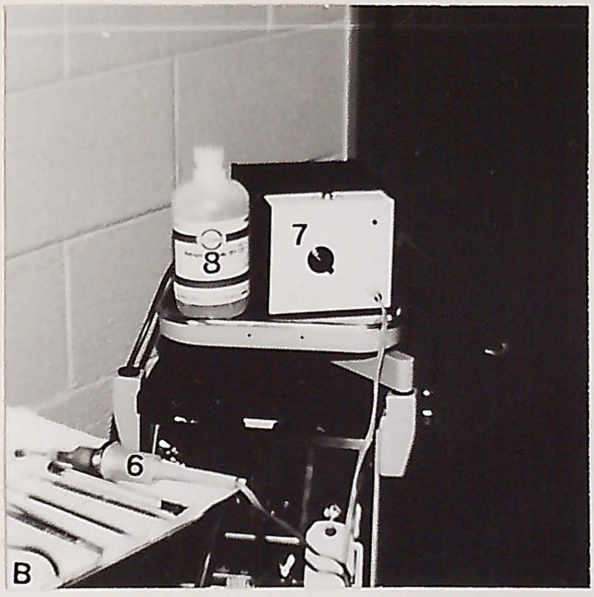
TABLE XX

Average thickness and range of thickness of the enamel in rabbit incisors at the central groove of the maxillary teeth and at the mid-point of the labial surface of the mandibular teeth. Measurements were made in the incisal, middle and gingival thirds of the incisors.

Location of Enamel	Average	Range
Maxillary Incisal Third	18.8 microns	10.0 to 18.0 microns
Maxillary Middle Third	19.0 microns	8.00 to 38.0 microns
Maxillary Gingival Third	17.3 microns	9.00 to 30.0 microns
Mandibular Incisal Third	36.1 microns	22.0 to 46.0 microns
Mandibular Middle Third	35.5 microns	28.0 to 44.0 microns
Mandibular Gingival Third	35.5 microns	28.0 to 44.0 microns

Figure 1. Photographs (A, B, C) of the instruments used during the bleaching of the rabbits' incisors. 1, Shade guides. 2, Rubber dam frame and instruments. 3, Petroleum jelly for sealing rubber dam. 4, Moistened pumice. 5, Thermometer. 6, Hand held heating instrument. 7, Control unit for heating instrument. 8, Bottle of 30 percent hydrogen peroxide. 9, Ultraviolet lamp for fluorescent slides. 10, Barrier filter and camera for fluorescent slides.


3
4
1
2
6
5
A


8
7
6
B

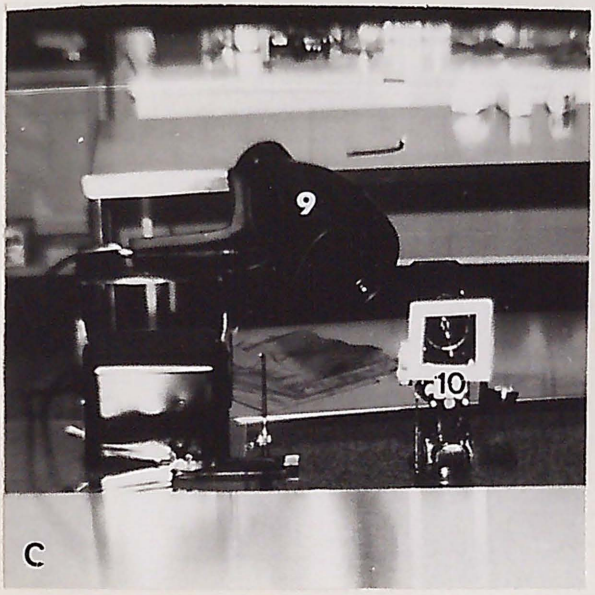

9
10
C

Figure 2. Photographs of the isolation of a mandibular rabbit incisor with the rubber dam.

A. Rubber dam isolating mandibular right incisor.

B. Close-up of the mandibular incisor and dam.

C. Isolated incisor with cotton pellets saturated with 30 percent hydrogen peroxide.


A


B

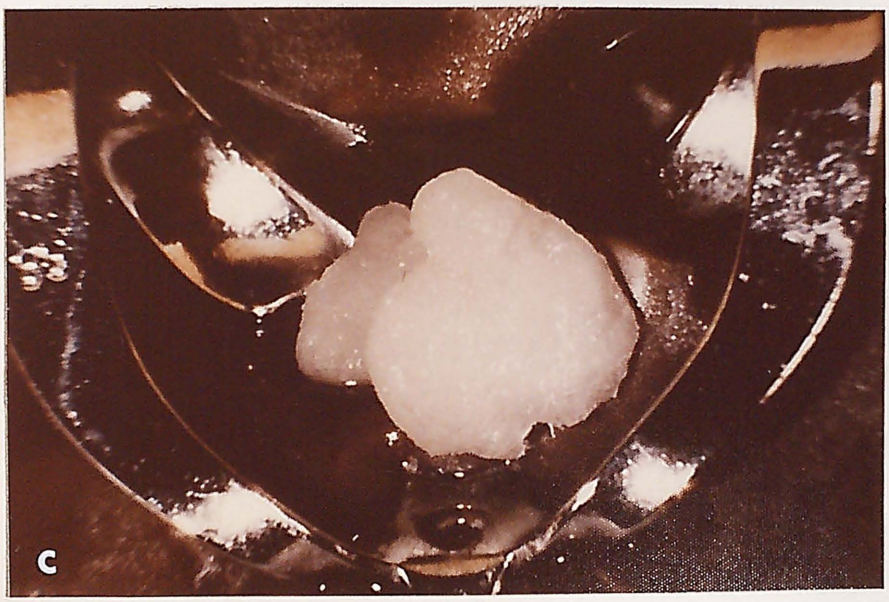
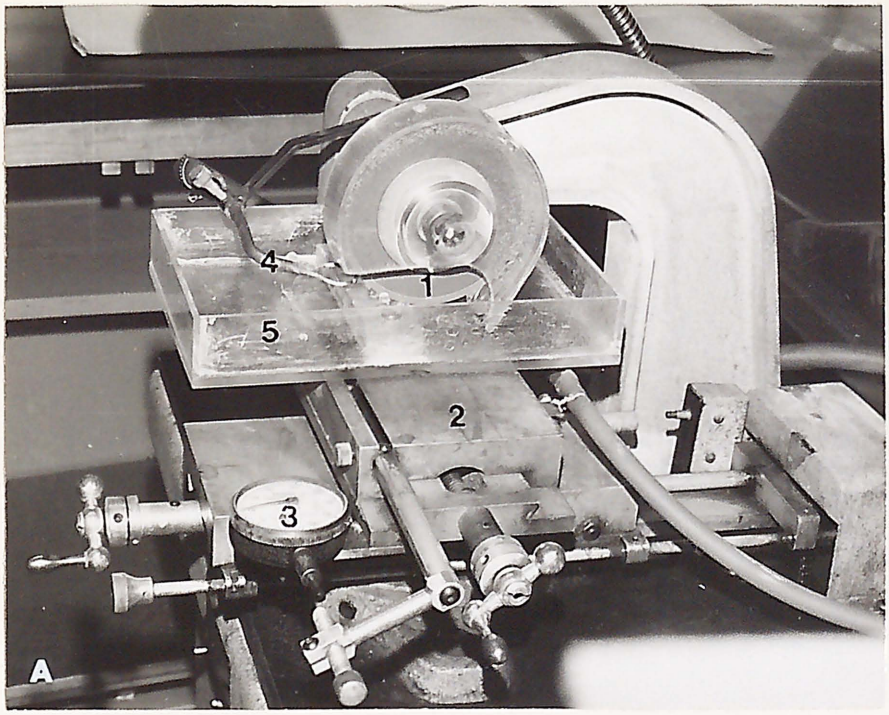

C

Figure 3. Two photographs (A, B) of the thin sectioning instrument used to prepare the transverse ground sections. 1, Diamond coated grinding wheel .015 inches thick. 2, Micro-feed table. 3, Micrometer for determining the width of the section. 4, Water coolent tube. 5, Sectioning jig fastened to micro-feed table. 6, Sectioned rabbit incisor in block of Bio-Plastic.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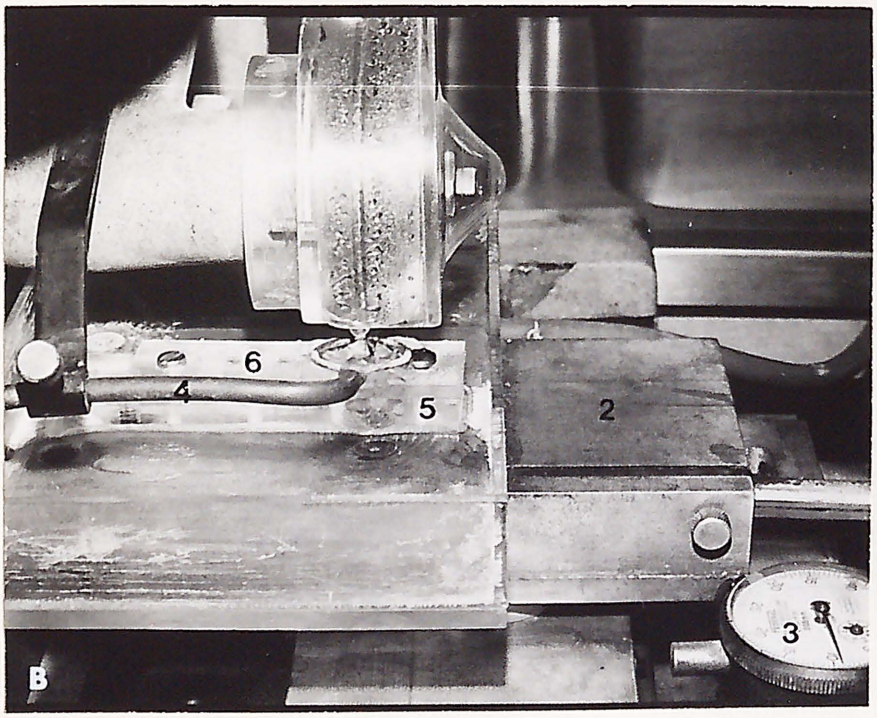
2
3
A


6
4
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B

Figure 4. A schematic block diagram of the television fluorescent intensity measurement instrumentation.

BLOCK DIAGRAM OF ELECTRONIC MEASURING EQUIPEMENT

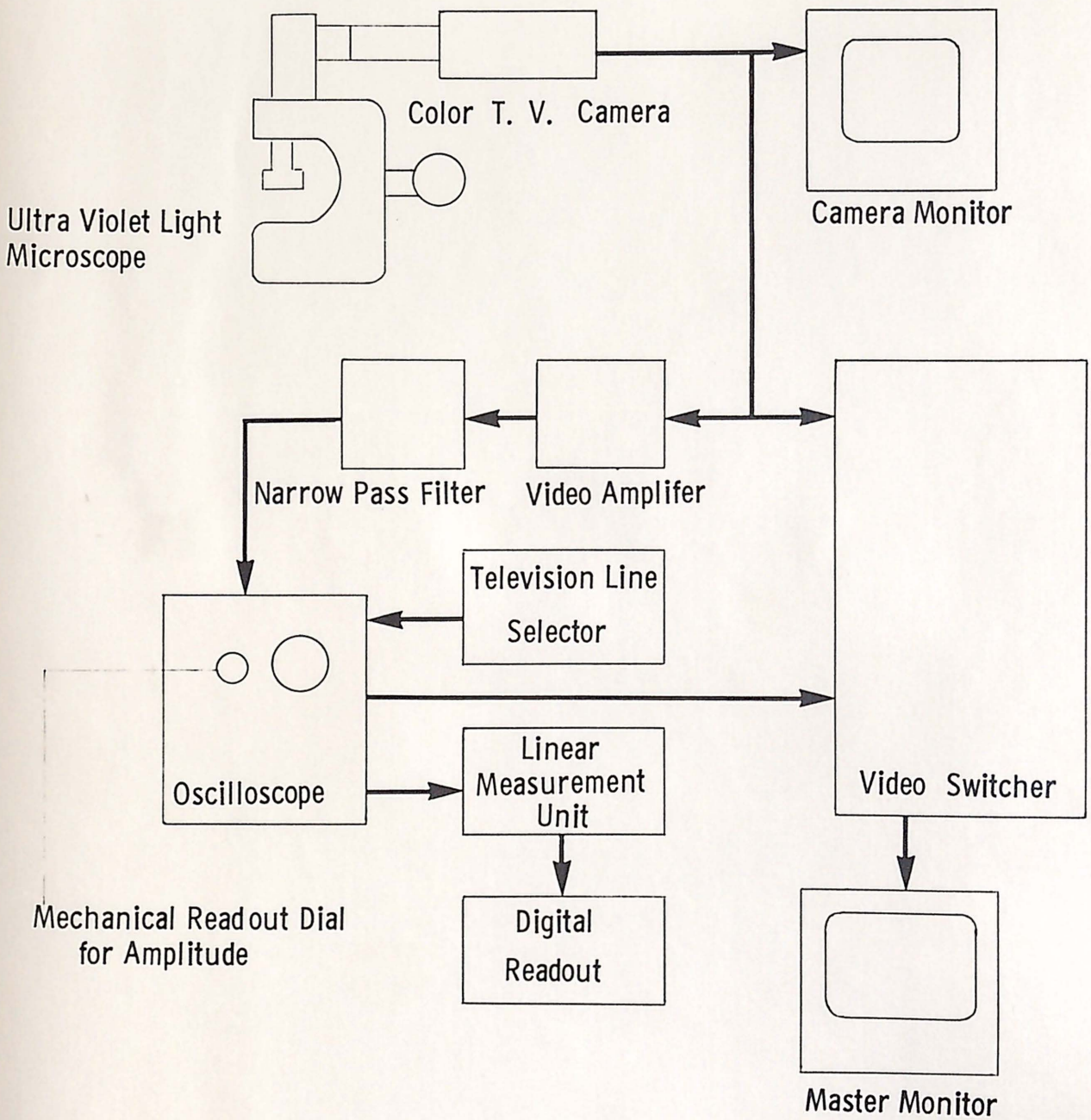
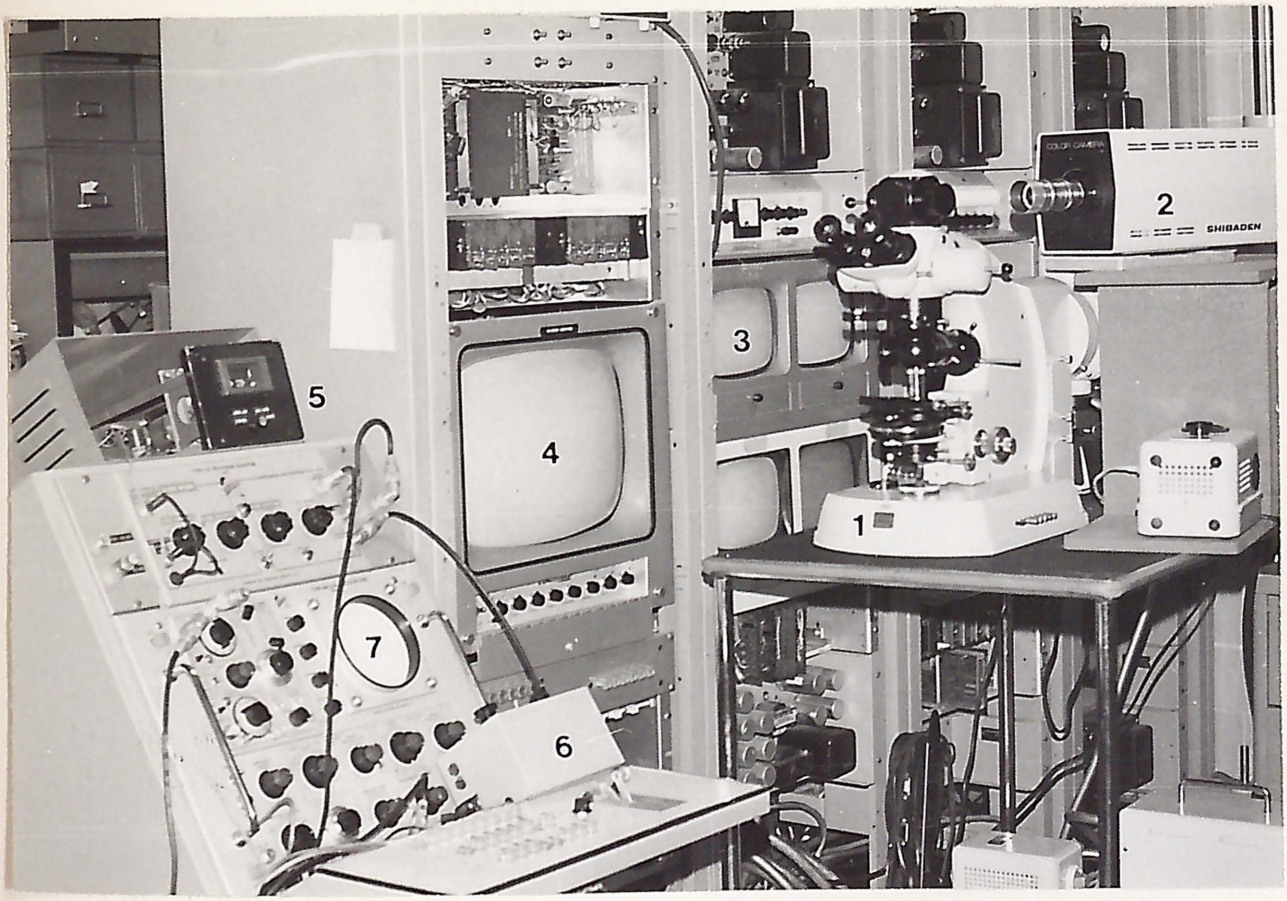


Figure 5. Photographs of the television fluorescent intensity measurement instrumentation and wave form of the video signal on the oscilloscope. 1, Ultraviolet light microscope. 2, Color television camera. 3, Camera monitor. 4, Master monitor. 5, Digital readout of linear measurement. 6, Calibration controls for linear measurement. 7, Oscilloscope screen. 8, Wave-form of the video signal of tetracycline fluorescence from ground section on oscilloscope screen. 8-A, Base line of signal. 8-B, Peak of electronic noise. 8-C, Peaks of the individual bands of fluorescing tetracycline in the ground section.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SHIBADEN
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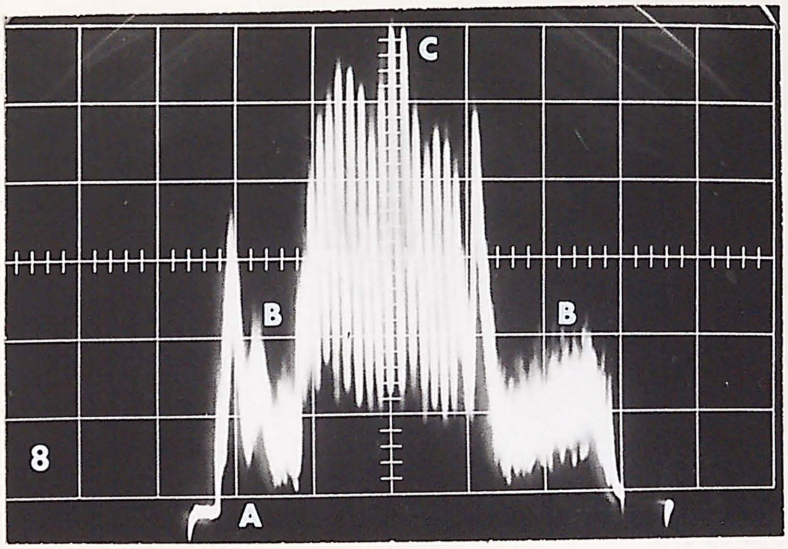
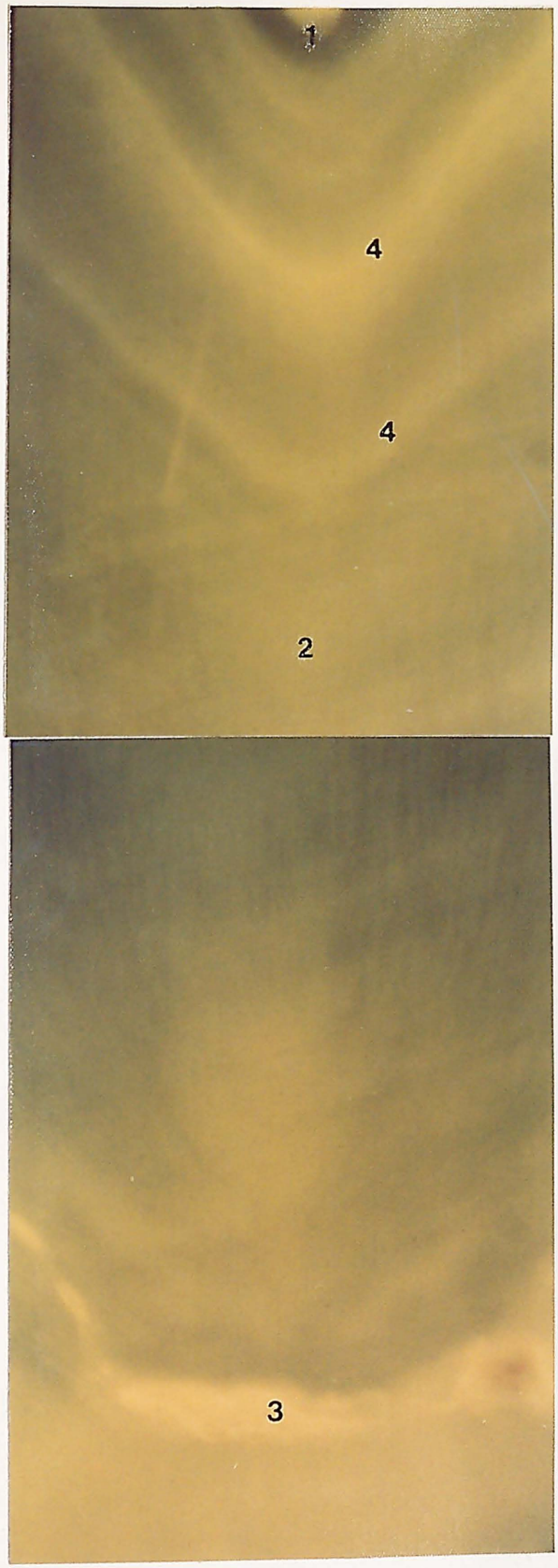

C
B
B
8
A

Figure 6. Composite photomicrograph of fluorescence from a ground section of an unstained maxillary rabbit incisor. The composite demonstrates a portion of a transverse ground section from the enamel (1) to the pulp canal (3). Strong autofluorescence is seen in the pulp canal (3) and undetermined fluorescence (4) is seen in the dentin (2). (Original magnification x 100--exposure 60 seconds)


1
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3

Figure 7. Composite photomicrograph of fluorescence from a ground section of an unstained mandibular rabbit incisor. The composite demonstrates a portion of a transverse ground section from the enamel (1) to the pulp canal (3). Strong autofluorescence is seen in the pulp canal (3) but not in the dentin (2). (Original magnification x 100--exposure 60 seconds)

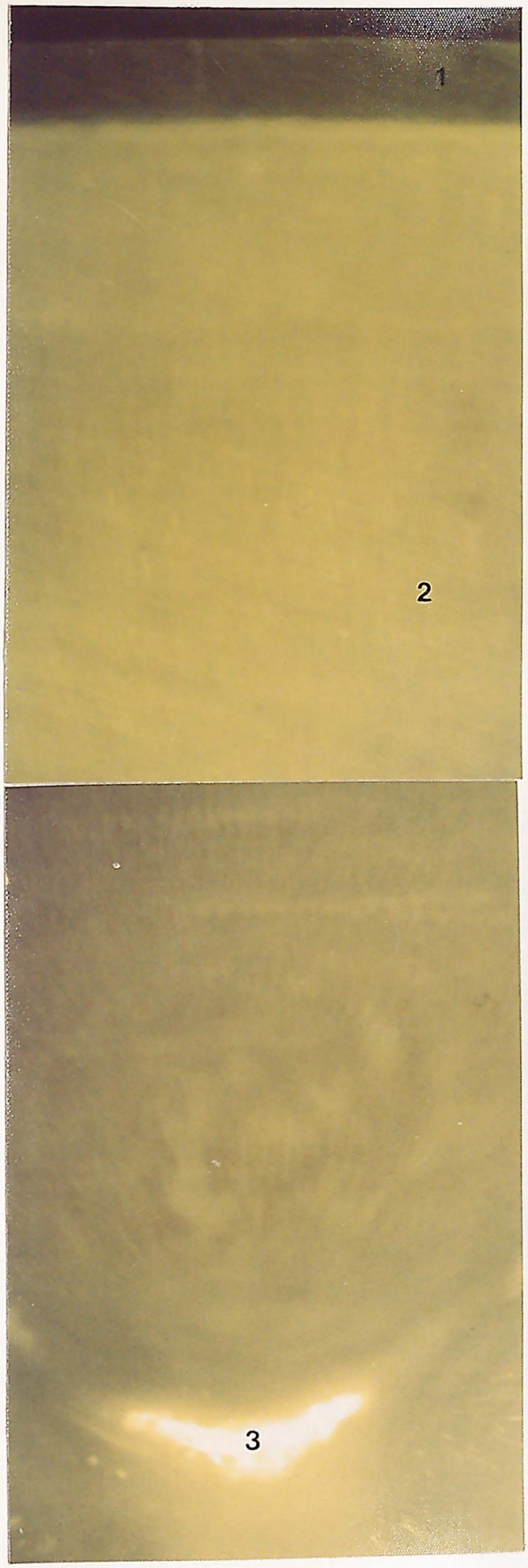
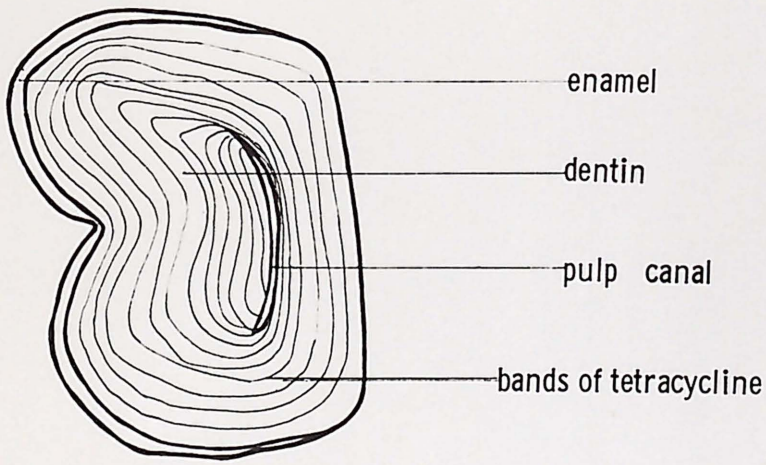
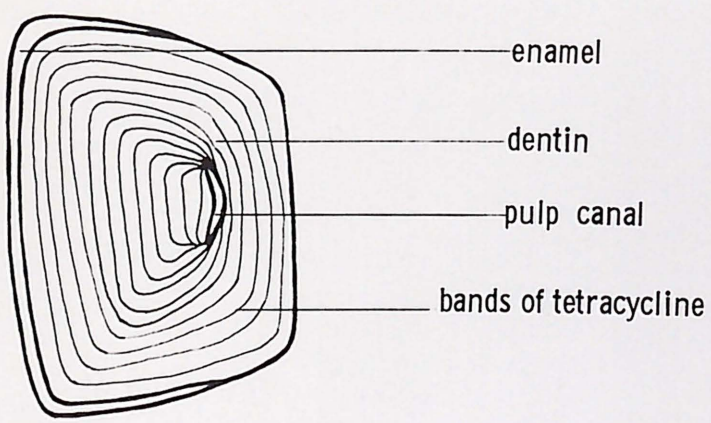

1
2
3

Figure 8. Diagram of transverse ground sections of maxillary and mandibular rabbit incisors with oxytetracycline stain. Note the difference in the shape of the two types of teeth and the difference in the enamel thickness.

DIAGRAM OF TRANSVERSE GROUND SECTIONS



MAXILLARY INCISOR



MANDIBULAR INCISOR

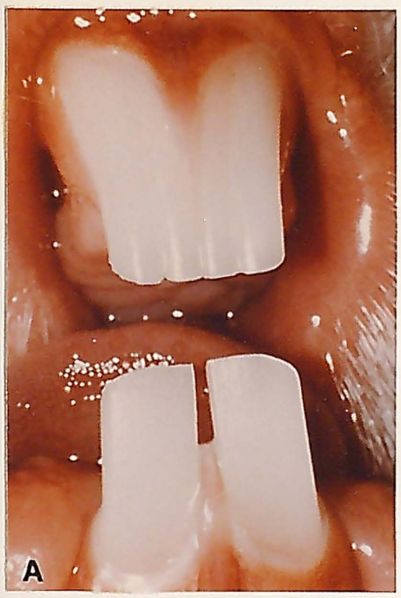
Figure 9. Clinical photographs of unstained and stained, unbleached and bleached rabbit incisors.

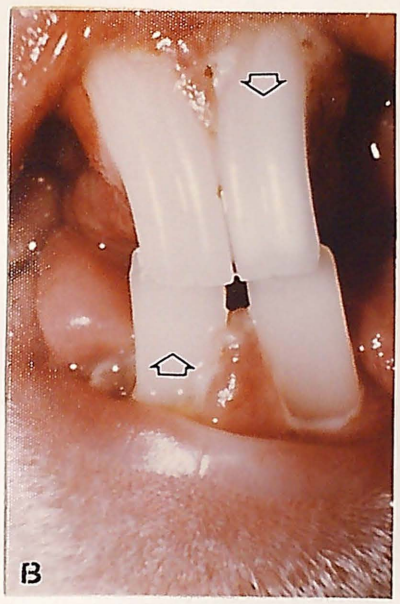
A. Received no oxytetracycline and not bleached

B. Received no oxytetracycline and incisors with arrows bleached twice

C. Received oxytetracycline and not bleached

D. Received oxytetracycline and incisors with arrows bleached twice.


A


B


C

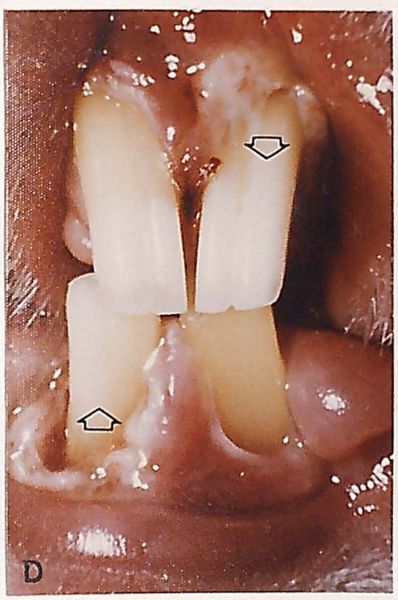

D

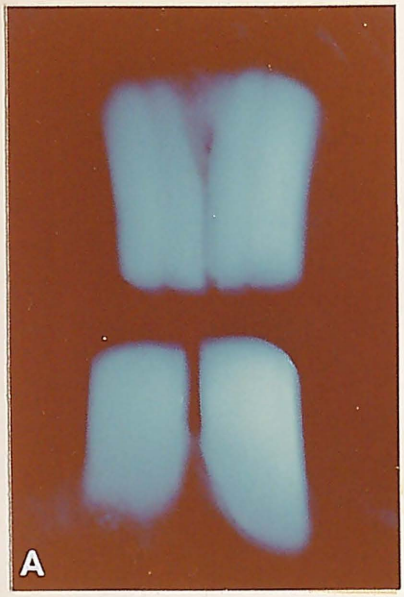
Figure 10. Photographs taken with ultraviolet light of macroscopic fluorescence in unstained and stained, unbleached and bleached rabbit incisors.

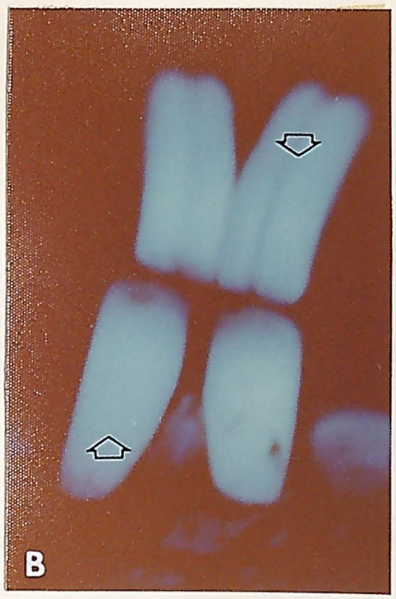
A. Received no oxytetracycline and not bleached

B. Received no oxytetracycline and incisors with arrows bleached twice

C. Received oxytetracycline and not bleached

D. Received oxytetracycline and incisors with arrows bleached twice


A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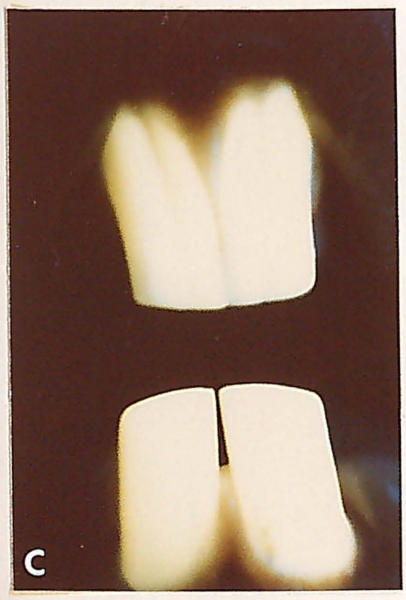
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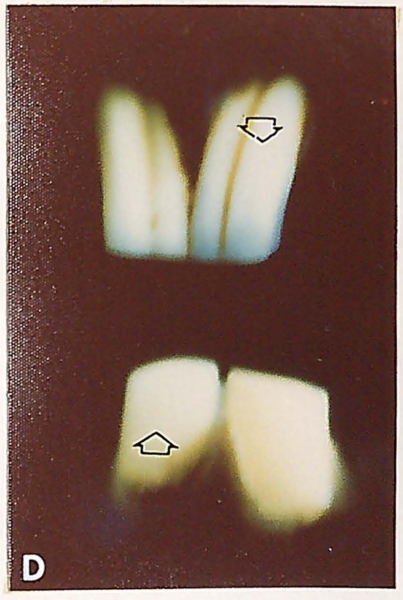
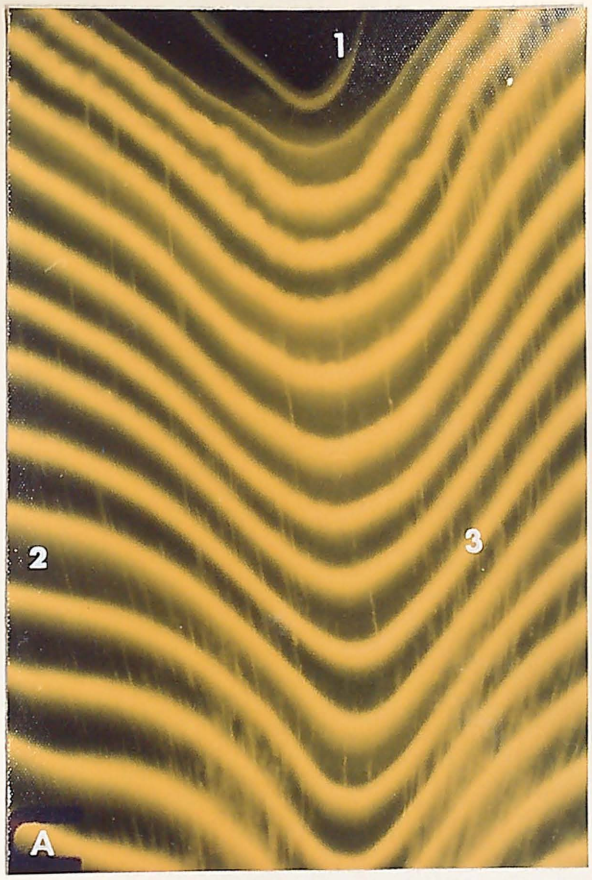

D

Figure 11. Photomicrographs of fluorescence from transverse ground sections of unbleached (A) and bleached (B) maxillary rabbit incisors with oxytetracycline. A comparison demonstrates a lowering of fluorescent intensity in enamel (1) and the bands of tetracycline (3) in dentin (2) after two bleaches. (Original magnification x 100 exposure 5 seconds)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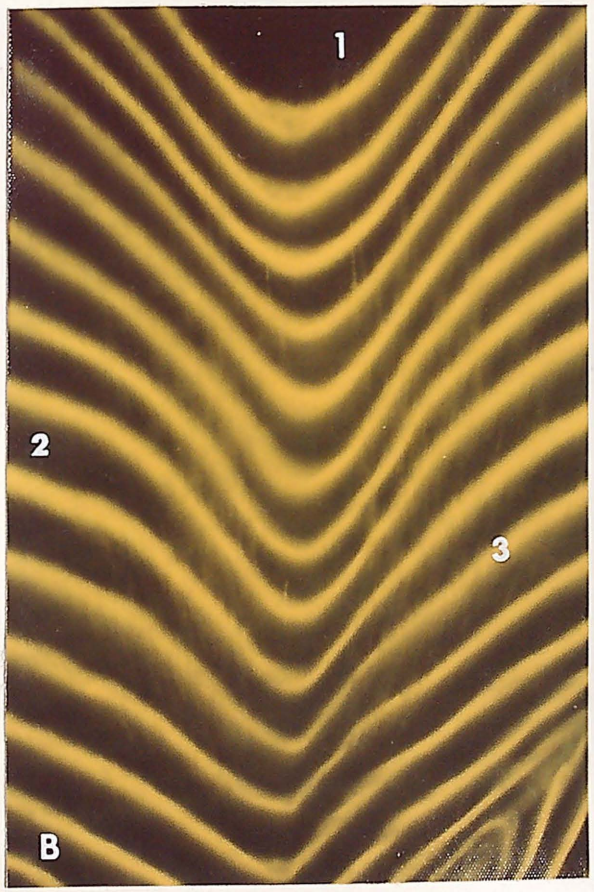
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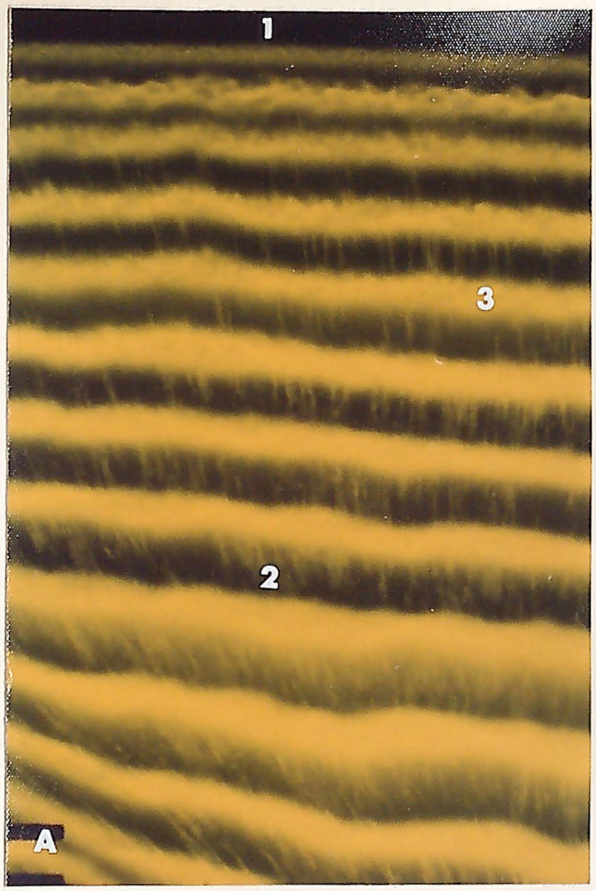
B

Figure 12. Photomicrographs of fluorescence from transverse ground sections of unbleached (A) and bleached (B) mandibular rabbit incisors with oxytetracycline. A comparison demonstrates a lowering of fluorescent intensity in enamel (1) and the bands of tetracycline (3) in dentin (2) after two bleaches. (Original magnification x 100 exposure 5 seconds)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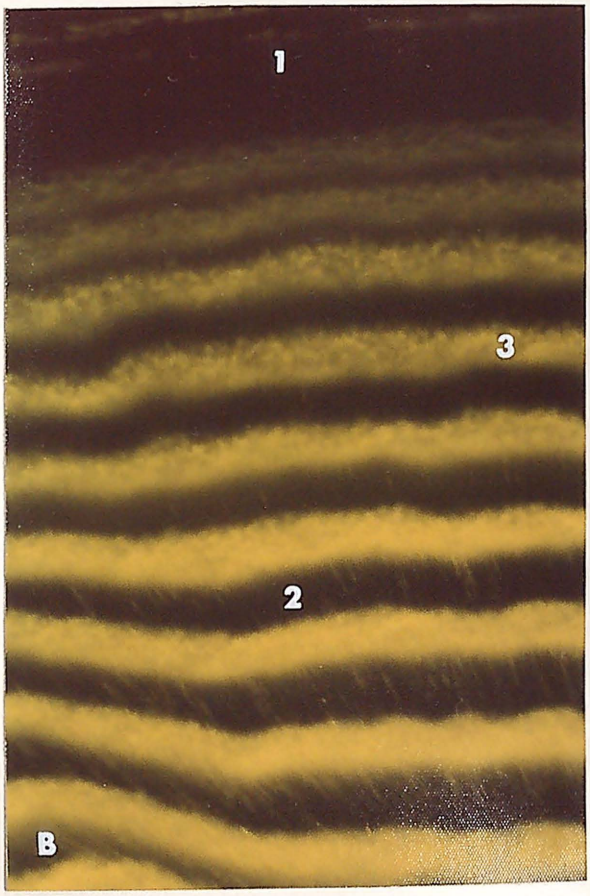
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DISCUSSION

This study was designed to investigate why the bleaching of tetracycline-stained teeth with the technique used by Cohen and Parkins[5] and Arens, Rich and Healy[6] was only partially successful in improving the esthetics of these teeth. This led to the hypothesis that the tetracycline fluorescence was lower in teeth demonstrating clinical bleaching of the stain, and that the bleaching effect was strongest at the dentino-enamel junction.

This study demonstrated that, as a group, tetracycline-stained teeth which were bleached showed a loss of tetracycline fluorescent intensity. The difference in intensity between unbleached and bleached tetracycline-stained teeth was not always statistically significant but it was measurable and apparent. This change in fluorescent intensity was in one direction only, and of such magnitude that bleached and unbleached tetracycline-stained teeth could be compared as a group for the effectiveness of bleaching.

The tetracycline-stained maxillary incisors which were bleached twice demonstrated a significant ($P \leq 0.001$) reduction in fluorescent intensity in the whole tooth, but only the incisal one-third of the tetracycline-stained mandibular incisors which were bleached twice showed a significant ($P \leq 0.005$) reduction in fluorescent intensity (Table XVIII). This difference in the bleaching effect between the two types of teeth may be related to one or more of the following factors: 1) The mandibular incisors were difficult to isolate and clamp with a rubber dam

clamp, resulting in less clinical crown being exposed; 2) the mandibular incisors were not as accessible to bleaching as they were anatomically behind the maxillary incisors; and 3) the enamel on the two types of teeth was different (Table XX). The enamel of the mandibular incisor was uniform in thickness as was on the average about 36 microns thick. The enamel of the maxillary incisor was grooved on the labial surface and the average thickness in this area, where the fluorescent intensities were measured, was about 18 microns.

The data indicate that teeth in the same arch of a rabbit could not be used for individual comparisons. The tetracycline-stained matched pairs of incisors used to determine whether they could serve as their own controls showed a significant difference in 18 of 36 pairs of teeth (Tables XII and XIII). It is possible that differences between individual teeth in the same arch were due to variations in the regional anatomy or in eruption and development, which caused them to absorb the oxytetracycline at varying rates. It is more likely; however, that the difference is due to errors of ground section preparation and fluorescent intensity measurement.

It is difficult to prepare nearly plano-parallel serial ground sections of 100 ± 5 microns from identical locations on each of the paired incisors. The vibration of the sectioning machine, the vibration of the room and varying room temperatures all offered possibilities for error. The independent investigator, used to eliminate bias in the selection of sections to be measured, selected specimens which were closest to being 100 microns and plano-parallel. The sections were selected from the same area (incisal, middle and gingival thirds), but not from

identical locations on each of the paired incisors. In some cases there was a difference of a millimeter from the incisal edge between the paired incisors. Therefore, the location of the same dose of tetracycline, which appeared as bands in the dentin, was at a different distance from the outer enamel surface in each of the paired ground sections.

Variability in the fluorescent intensity seen in the ground sections was also the result of fluctuations in the intensity of the ultraviolet light on the microscope, even after it was stabilized with a voltage regulator. Variations in the dental school building current also caused oscilloscope measurement error of the fluorescent intensity. During normal operation of the building elevators and heavy electronic machinery, there was a 20 to 40 unit deflection of the oscilloscope. Table III demonstrates this error in the middle third group which shows a significant difference at the < 0.05 level of confidence. These measurements were made during the day and the peak values for fluorescent intensity were due to extraneous electronic noise. After this fact was discovered, the deviation was reduced to about 16 units by making the measurements at night and on week-ends when the building elevators were not used.

For whatever reason, the variation in the fluorescent intensities of tetracycline-stained contralateral teeth implies that no autocontrol measurements can be used in comparing the individual paired teeth from the same jaw.

During the measurement procedures, it was noted that when a ground section showing tetracycline stain was repeatedly exposed to ultraviolet light, the fluorescent intensity of the specimen decreased. This reduction

in fluorescent intensity seemed to occur more rapidly in those ground sections from incisors which were bleached. The possibility arises that ultraviolet light was disrupting the bonds in individual molecules of tetracycline, or disrupting the relationship of the tetracycline to the binding site, or otherwise altering the chemistry of the stained tissue. Therefore the use of ultraviolet light in the measurement procedure should be evaluated.

The observation that exposure to ultraviolet light reduced the ability of tetracycline to fluoresce may have an application clinically. Perhaps the use of ultraviolet light in conjunction with the present bleaching procedure would bleach tetracycline-stained teeth more effectively. Therefore, the use of ultraviolet light with heat and thirty percent hydrogen peroxide should be evaluated for bleaching effectiveness.

Microscopic examination of the ground sections with tetracycline showed that although the fluorescent intensity was lower in those teeth which were bleached, the tetracycline bands still fluoresced (Figures 11 and 12); and although the bleaching-effect penetrated to the pulp canal, the strongest bleaching occurred in the dentin up to 150 to 350 microns from the outer enamel surface.

This may explain why Cohen and Parkins[5] and Arens, Rich and Healey[6] indicated that tetracycline-stained teeth having a gray hue did not bleach as well as those teeth with a yellow or brown hue. In some cases the authors also noted a return of the stain after bleaching and in these cases additional bleaching was required. Brearley and Storey[160] presented evidence that tetracycline deposits in teeth turned from yellow to brown when exposed to sunlight. They demonstrated that teeth with a yellow or brown

stain (which are easier to bleach) had tetracycline in the dentin at or very close to the dentino-enamel junction. Teeth with gray hues (the most difficult to bleach) had tetracycline deposits in the dentin furthest from the dentino-enamel junction. They attributed the gray hue of tetracycline to light reflecting off brown stain deep in the dentin and then being masked by the white of the enamel and the lighter yellow of unaffected dentin. This combination produced the gray colors.

Thus the persistence of the gray stain even after several bleachings could be related to the stain's position in the tooth, and the fact that the bleach may not be penetrating to the tetracycline. In those teeth which were bleached and the stain returned, it may be that the tetracycline was not completely removed from the dentin.

Bennett and Law[186] and Bennett[187] stated that enamel contained tetracycline. Their chemical analysis showed that tetracycline deposited in enamel and dentin was in a ratio of 1 to 9. This study indicates that the fluorescent intensity of tetracycline was in a ratio nearer 1 to 20, as represented by 10 to 15 units in enamel and 200 to 300 units in dentin. The fluorescent intensity of tetracycline in the enamel was faint, diffuse and very difficult to measure. It could have been caused by the transmission through the enamel of fluorescent light from the highly fluorescing dentin. Since the intensity of tetracycline fluorescence was so faint, the measurement of fluorescence in enamel does not appear to be a good method of determining the presence or absence of tetracycline in enamel.

There were several technical problems encountered in the study. The rabbit is a very fragile and sensitive animal, and several anesthetic deaths occurred with anesthetic doses below those normally given because

the rabbits became excited during clinical preparation. The fact that the incisors were continually growing prevented more than two bleaches per rabbit. Therefore, a better animal would be a primate. The primate's teeth would be more comparable to human teeth and the teeth could be bleached until the desired amount of clinical color change was obtained.

The administration of oral oxytetracycline to the rabbits in their water was less than satisfactory for the following reasons: 1) The rabbits did not drink water at a uniform rate, 2) the oxytetracycline in the pediatric syrup precipitated out on standing after it had been in the water bottle for several hours, 3) the oral dose is equivalent in effect to about one-fourth the parenteral dose.

The rabbits' teeth also grew at varying rates. The maxillary incisors grew about 20 to 30 microns every three days and mandibular incisors about 30 to 40 microns over the same period. The macroscopic tetracycline fluorescence first appeared in the mandibular incisors after about three weeks on the drug. It required about 8 to 9 weeks for all the incisors to stain completely.

The control rabbits which were not to receive the tetracycline may have received minute amounts of the drug accidentally. Figure 6 shows either very strong auto-fluorescence or very weak bands of tetracycline fluorescence. This fluorescence does not appear in the mandibular incisor of the same animal (Figure 7). Its intensity was also too weak to be measured by our instrumentation. If tetracycline contamination did occur it could have happened when the rabbits' cages were cleaned and sterilized. During sterilization and cooling of the cages, the rabbits with their water supply were moved to separate holding cages. These cages were common to

all rabbits and a rabbit who was not receiving tetracycline might eat the feces of an animal which had received the drug, or he might lick dried OTC from the cage bars which could have dripped from a water bottle of another rabbit previously held in that cage. This problem probably could have been avoided by placing the experimental and control animals in separate rooms during the study.

Photographic slides of the clinical bleaching process were taken with only one camera system. This system provided a simple method, and the only one available, to take both pre-operative and post-operative slides in white and ultraviolet light. Better results might have been obtained by using an ultraviolet electronic flash. The use of exciter filters over a 200 watt-second flash source and Hi-Speed Ektachrome increased to 400 ASA by processing, might yield more reproducible and less distorted slides of the fluorescing image. This subject is explained in great detail by Gibson[275] and the Kodak Publication M-27.[190]

The measurement instrumentation could be improved since electronic capabilities are constantly changing. Currently, oscilloscopes are available which draw far less current than those used in this study, due to solid state circuitry. This type of oscilloscope would be less likely to vary with the current load in the building, which becomes critical when measuring the intensity deflection on the order of 5/1000 of a volt. Stop-action video recording instrumentation can allow the researcher to freeze the signal being studied and measure it more accurately. The fluctuation of the light source on the ultraviolet light microscope can be eliminated by the use of circuitry which automatically compensates for the video signal received by the television camera for measurement.

In all aspects the experiment was an exploratory study. The use of electronics in dental research is encouraged, and it is hoped that future research will find a more effective way of bleaching tetracycline-stained teeth. It should be remembered that the bleach is effective for only about the first 200 microns and that the tetracycline fluorescence is not totally removed. The use of ultraviolet light in conjunction with the present method of bleaching should be evaluated. Also, the minimal fluorescence of tetracycline in enamel does not accurately indicate its absence from enamel.

SUMMARY AND CONCLUSIONS

Nineteen male New Zealand white rabbits, with 58 incisors stained with oxytetracycline and 16 incisors as unstained controls, were used to determine the effectiveness of bleaching tetracycline stained teeth with 30 percent hydrogen peroxide and heat. Tetracycline staining was induced by oxytetracycline (5.0 mg./lb. of body weight) daily in the rabbits' water supply and subcutaneous injections of oxytetracycline (5.0 mg./lb. of body weight) every three days. The four control rabbits received no oxytetracycline. The drug was discontinued after eight weeks and three rabbits were sacrificed to ascertain whether the tetracycline stain was comparable in teeth of the same jaw. The remaining 16 animals had one maxillary incisor bleached and one mandibular incisor bleached with 30 percent hydrogen peroxide and heat for 10 minutes per tooth. The contralateral tooth was protected from bleaching by the rubber dam. The animals were sacrificed 24 hours after the last bleach.

Serial ground sections approximately plano-parallel 100 ± 5 microns thick were made of the following contralateral pairs of incisors: 1) four pairs with no oxytetracycline, bleached once; 2) four pairs with no oxytetracycline, bleached twice; 3) eight pairs with oxytetracycline, bleached once; and 4) 15 pairs with oxytetracycline, bleached twice.

The fluorescent intensity of 374 selected ground sections in the incisal, middle and gingival thirds of the teeth was measured using an ultraviolet light microscope coupled to a television electronic measurement

system. These measurements were statistically analyzed by t-test, and observations were correlated. The following conclusions were reached:

1. The dentin of tetracycline-stained maxillary incisors, bleached twice, had significantly lower tetracycline fluorescent intensity ($P \leq 0.001$) than the dentin of unbleached tetracycline-stained maxillary incisors.

2. The dentin in the incisal one-third of tetracycline-stained mandibular incisors, bleached twice, had significantly lower tetracycline fluorescent intensity ($P \leq 0.005$) than the tetracycline-stained mandibular incisors which were not bleached.

3. The greatest bleaching effect (loss of fluorescent intensity) of tetracycline-stained teeth occurred in the dentin closest to the enamel surface of the tooth. This effect occurred to a depth of about 250 to 350 microns from the outer enamel surface in the maxillary incisors, and about 150 to 250 microns from the outer enamel surface in the mandibular incisors.

4. The comparison of the pre-operative and post-operative clinical Kodachrome slides taken in white and ultraviolet light indicate that the loss of tetracycline pigment from the tooth was associated with the loss of tetracycline fluorescent intensity.

5. The dentin of tetracycline-stained teeth, bleached once, demonstrated a tendency towards less fluorescence than those teeth that were not bleached.

6. The fluorescence of tetracycline-stained dentin in the rabbit incisors was never totally removed by the bleaching process. In

all groups of teeth the tetracycline fluorescent intensity was lower but the fluorescent stain persisted.

7. The bleaching procedure did not alter the primary fluorescence of the teeth not stained with tetracycline.

8. The fluorescence of the tetracycline-stained rabbit incisors was greater than the unstained rabbit incisors.

9. The fluorescent intensity of dentin was significantly greater ($P \ll 0.01$) than the enamel in tetracycline-stained rabbit incisors.

10. The intensity of tetracycline fluorescence in enamel was negligible. Therefore, it is questionable whether the presence of tetracycline in enamel can be demonstrated by fluorescent intensity.

11. The ultraviolet light used in the measurement procedure reduced the fluorescent intensity in tetracycline-stained teeth.

APPENDIX I

The computer print-out demonstrates the fluorescent intensity of of a right incisor (I1) and a left incisor (I2) in pairs of teeth which have not been bleached. In pairs of teeth which have been bleached, I1 is the unbleached tooth and I2 is the bleached tooth. I3 is the difference between the two teeth (I1 - I2). Each number in Column 1 or Column 2 represents the intensity of tetracycline fluorescence for a specific band of tetracycline in dentin. The intensity was determined by subtracting from the peaks of the wave form on the oscilloscope the base of the wave and the peak of the electronic noise (Figure 5). The units for intensity indicate 1/1000 of the total oscilloscope height. Due to amplification of the signal and modification of the oscilloscope, no absolute unit value could be assigned.

Columns D1 and D2 represent the distance in microns from the outer enamel surface to the leading edge of the band of tetracycline. D1 corresponds to the I1 column and D2 corresponds to the I2 column.

The rabbit number is given at the end and "N" represents the number of bands of tetracycline in that ground section.

The mean, standard deviation, and standard error were then computed for each column and their number (SD1) corresponds to the "I" column numbers.

MAXILLARY INCISAL THIRD
STAINED, NO BLEACH

I1	I2	I3	D1	D2
109	199	-90	186	238
201	185	16	226	268
212	185	27	260	296
234	193	41	301	318
203	212	-9	334	342
187	195	-8	368	376
181	202	-21	393	410
181	185	-4	412	442
173	205	-32	437	476

RABBIT 10A N= 9

MEAN I1= 186.778 MEAN I2= 195.667 MEAN DIF= -8.88889
SD1= 34.7087 SD2= 9.70824 SD3= 38.2114
SE1= 11.5696 SE2= 3.23608 SE3= 12.7371

I1	I2	I3	D1	D2
214	216	-2	214	210
214	211	3	251	239
224	201	23	293	273
234	213	21	333	299
235	212	23	364	326
211	207	4	394	352
229	212	17	432	382
212	216	-4	458	413
230	191	39	474	442

RABBIT 10B N= 9

MEAN I1= 222.556 MEAN I2= 208.778 MEAN DIF= 13.7778
SD1= 9.85027 SD2= 8.12062 SD3= 14.3421
SE1= 3.28342 SE2= 2.70687 SE3= 4.78068

I1	I2	I3	D1	D2
242	247	-5	15	32
251	247	4	33	50
266	260	6	61	72
274	269	5	94	104
271	277	-6	127	133
268	283	-15	160	160
324	307	17	183	189
293	296	-3	217	219
282	316	-34	248	252
264	301	-37	280	281
287	339	-52	310	309
297	298	-1	338	340
260	297	-37	369	372
276	253	23	403	397
226	237	-11	431	418
239	247	-8	458	429

RABBIT 13A N= 16

MEAN I1= 270 MEAN I2= 279.625 MEAN DIF=-9.625
SD1= 24.3009 SD2= 29.6623 SD3= 20.8034
SE1= 6.07522 SE2= 7.41557 SE3= 5.20086

I1	I2	I3	D1	D2
207	165	42	13	12
228	192	36	38	38
240	221	19	56	60
283	216	67	82	84
267	238	29	114	114
269	244	25	144	145
331	231	100	173	177
311	328	-17	205	204
307	282	25	238	234
327	344	-17	269	267
320	286	34	300	303
329	289	40	326	339
307	293	14	354	373
314	271	43	380	403
284	277	7	408	432
274	221	53	428	454
283	274	9	450	468

RABBIT 13B N= 17

MEAN I1= 287.118 MEAN I2= 257.176 MEAN DIF= 29.9412
SD1= 36.6723 SD2= 47.0694 SD3= 28.5908
SE1= 8.89435 SE2= 11.416 SE3= 6.93429

I1	I2	I3	D1	D2
189	254	-65	252	252
213	259	-46	291	287
236	169	67	324	350
251	260	-9	352	361
216	258	-42	380	390
175	210	-35	430	449

RABBIT 15A N= 6

MEAN I1= 213.333 MEAN I2= 235 MEAN DIF=-21.6667
SD1= 28.289 SD2= 37.6085 SD3= 47.0815
SE1= 11.5489 SE2= 15.3536 SE3= 19.2209

I1	I2	I3	D1	D2
187	198	-11	270	312
195	191	4	310	352
225	191	34	349	388
210	224	-14	379	421
214	183	31	410	448
179	161	18	431	472
157	188	-31	452	482

RABBIT 15B N= 7

MEAN I1= 195.286 MEAN I2= 190.857 MEAN DIF=
4.42857
SD1= 23.2717 SD2= 18.7566 SD3= 24.4871
SE1= 8.79587 SE2= 7.08932 SE3= 9.25526

MAXILLARY MIDDLE THIRD
STAINED, NO BLEACH

I1	I2	I3	D1	D2
210	305	-95	156	218
195	273	-78	194	248
209	292	-83	229	288
229	338	-109	261	322
230	298	-68	294	352
205	279	-74	325	382
192	248	-56	356	412
202	243	-41	381	381
187	243	-56	408	455
169	235	-66	426	481

RABBIT 10A N= 10

MEAN I1= 202.8 MEAN I2= 275.4 MEAN DIF= -72.6
SD1= 18.5341 SD2= 33.4704 SD3= 19.9455
SE1= 5.86098 SE2= 10.5843 SE3= 6.30732

I1	I2	I3	D1	D2
328	325	3	90	179
313	318	-5	132	216
315	303	12	170	248
333	335	-2	202	278
310	324	-14	236	312
309	297	12	270	348
318	277	41	290	374
323	289	34	314	402
297	263	34	344	418
282	265	17	370	439
261	256	5	395	451

RABBIT 10B N= 11

MEAN I1= 308.091 MEAN I2= 295.636 MEAN DIF= 12.4545
SD1= 21.0592 SD2= 27.8182 SD3= 17.6712
SE1= 6.34959 SE2= 8.38752 SE3= 5.32808

I1	I2	I3	D1	D2
156	228	-72	16	28
291	236	55	41	49
303	245	58	64	74
320	257	63	90	98
355	312	43	112	123
341	281	60	140	153
316	273	43	168	184
335	290	45	193	212
335	281	54	220	240
359	305	54	244	263
330	289	41	270	294
358	300	58	298	321
350	275	75	327	348
350	269	81	357	390
325	260	65	382	397
315	220	95	408	408

RABBIT 13A N= 16

MEAN I1= 321.188 MEAN I2= 270.063 MEAN DIF= 51.125

SD1= 48.3394 SD2= 27.3751 SD3= 35.9238

SE1= 12.0848 SE2= 6.84377 SE3= 8.98094

I1	I2	I3	D1	D2
275	173	102	27	20
280	234	46	49	43
313	251	62	73	67
354	257	97	98	91
332	259	73	121	121
316	323	-7	153	146
294	257	37	181	175
306	260	46	206	205
342	272	70	231	233
300	254	46	257	262
328	292	36	283	289
302	273	29	313	317
304	308	-4	338	346
325	276	49	364	374
282	256	26	390	395
252	197	55	412	415

RABBIT 13B N= 16

MEAN I1= 306.563 MEAN I2= 258.875 MEAN DIF= 47.6875

SD1= 26.5756 SD2= 36.7076 SD3= 30.0238

SE1= 6.6439 SE2= 9.17691 SE3= 7.50595

I1	I2	I3	D1	D2
264	219	45	190	178
265	240	25	231	218
290	231	59	270	255
296	254	42	302	293
278	252	26	338	324
287	249	38	371	356
286	232	54	399	386
253	217	36	425	414
278	212	66	452	434
244	175	69	470	452
255	194	61	482	468

RABBIT 15A N= 11

MEAN I1= 272.364 MEAN I2= 225 MEAN DIF= 47.3636
SD1= 17.1422 SD2= 24.8717 SD3= 15.4549
SE1= 5.16856 SE2= 7.49909 SE3= 4.65983

I1	I2	I3	D1	D2
229	231	-2	206	116
249	230	19	248	152
290	244	46	281	182
264	253	11	318	212
274	250	24	353	250
258	261	-3	386	290
278	263	15	414	324
218	247	-29	435	354
208	276	-68	458	384
210	236	-26	474	408
214	234	-20	480	440

RABBIT 15B N= 11

MEAN I1= 244.727 MEAN I2= 247.727 MEAN DIF=-3
SD1= 30.0669 SD2= 14.7519 SD3= 31.3911
SE1= 9.06551 SE2= 4.44786 SE3= 9.46477

MAXILLARY GINGIVAL THIRD
STAINED, NO BLEACH

I1	I2	I3	D1	D2
135	109	26	15	29
197	223	-26	42	66
203	243	-40	71	93
205	243	-38	104	132
200	268	-68	148	180
201	253	-52	184	220
206	229	-23	257	284
211	267	-56	296	312
181	217	-36	327	340
182	254	-72	359	366
190	247	-57	384	392
166	205	-39	412	417
153	188	-35	435	436
153	190	-37		
			456	478
158	197	-39	476	488

RABBIT 10A N= 15

MEAN I1= 182.733 MEAN I2= 222.2 MEAN DIF= -39.4667
SD1= 24.0192 SD2= 41.0317 SD3= 22.9747
SE1= 6.20174 SE2= 10.5943 SE3= 5.93205

I1	I2	I3	D1	D2
232	293	-61	38	88
231	287	-56	72	123
254	287	-33	114	163
278	317	-39	148	201
272	290	-18	224	265
260	280	-20	254	294
265	287	-22	286	321
250	247	3	319	347
232	260	-28	347	377
208	218	-10	370	395
218	242	-24	392	420
205	225	-20	414	442
171	200	-29	430	458
172	182	-10	444	468
170	199	-29	454	478

RABBIT 10B N= 15

MEAN I1= 227.867 MEAN I2= 254.267 MEAN DIF= -26.4
SD1= 36.6526 SD2= 41.5924 SD3= 16.6296
SE1= 9.46365 SE2= 10.7391 SE3= 4.29374

I1	I2	I3	D1	D2
152	139	13	19	19
214	218	-4	47	42
249	175	74	64	65
212	187	25	87	88
213	189	24	111	118
216	183	33	140	148
220	204	16	167	172
275	212	63	193	202
245	218	27	201	232
265	206	59	248	260
274	218	56	273	288
266	237	29	302	319
291	240	51	330	348
263	229	34	357	374

RABBIT 13A N= 14

MEAN I1= 239.643 MEAN I2= 203.929 MEAN DIF= 35.7143
SD1= 37.0293 SD2= 27.2974 SD3= 21.949
SE1= 9.8965 SE2= 7.29554 SE3= 5.86611

I1	I2	I3	D1	D2
172	84	88	28	21
207	91	116	50	48
202	153	49	69	71
199	167	32	92	94
218	180	38	118	122
222	205	17	148	152
254	192	62	173	181
228	212	16	204	213
253	211	42	236	242
251	215	36	263	274
254	244	10	292	303
290	218	72	320	333
260	214	46	342	361

RABBIT 13B N= 13

MEAN I1= 231.538 MEAN I2= 183.538 MEAN DIF= 48
SD1= 32.1989 SD2= 48.7743 SD3= 30.2297
SE1= 8.93037 SE2= 13.5276 SE3= 8.3842

I1	I2	I3	D1	D2
205	174	31	122	112
191	177	14	161	148
222	203	19	195	185
216	221	-5	227	218
230	192	38	263	252
228	200	28	304	290
239	204	35	335	324
225	196	29	368	354
241	213	28	400	386
208	187	21	429	414
220	200	20	454	440
203	183	20	470	462
202	184	18	493	482

RABBIT 15A N= 13

MEAN I1= 217.692 MEAN I2= 194.923 MEAN DIF= 22.7692.

SD1= 15.1788 SD2= 13.8411 SD3= 10.9784

SE1= 4.20986 SE2= 3.83884 SE3= 3.04487

I1	I2	I3	D1	D2
223	198	25	142	152
234	208	26	181	184
259	184	75	216	220
240	192	48	250	261
281	185	96	286	292
236	180	56	320	324
240	210	30	352	357
200	190	10	378	388
233	209	24	408	414
205	177	28	432	441
204	190	14	454	464
194	197	-3	473	482

RABBIT 15B N= 12

MEAN I1= 229.083 MEAN I2= 193.333 MEAN DIF= 35.75

SD1= 25.6531 SD2= 11.2761 SD3= 28.3007

SE1= 7.40542 SE2= 3.25514 SE3= 8.16972

MANDIBULAR INCISAL THIRD
STAINED, NO BLEACH

I1	I2	I3	D1	D2
181	216	-35	96	142
196	210	-14	131	178
215	215	0	167	218
231	238	-7	197	256
234	239	-5	236	286
212	231	-19	272	328
198	225	-27	304	354
216	206	10	334	369
207	176	31	373	396

RABBIT 10A N= 9

MEAN I1= 210 MEAN I2= 217.333 MEAN DIF=-7.33333
SD1= 16.8523 SD2= 19.4936 SD3= 19.8809
SE1= 5.61743 SE2= 6.49786 SE3= 6.62697

I1	I2	I3	D1	D2
192	209	-17	113	129
199	219	-20	148	164
216	228	-12	183	198
231	243	-12	222	232
221	237	-16	262	268
224	233	-9	292	306
223	220	3	328	340
233	227	6	364	370
220	202	18	398	405
221	197	24	426	433
206	206	0	452	455

RABBIT 10B N= 11

MEAN I1= 216.909 MEAN I2= 220.091 MEAN DIF=
-3.18182
SD1= 12.841 SD2= 15.0695 SD3= 14.6548
SE1= 3.8717 SE2= 4.54364 SE3= 4.41859

I1	I2	I3	D1	D2
80	176	-96	36	43
190	183	7	50	63
222	248	-26	66	84
273	237	36	88	108
254	352	-98	120	138
292	281	11	148	174
294	274	20	180	215
325	299	26	214	258
289	260	29	251	292
316	284	32	290	330
303	276	27	331	368
297	264	33	331	398
311	251	60	404	421

RABBIT 13A N= 13

MEAN I1= 265.077 MEAN I2= 260.385 MEAN DIF= 4.69231
SD1= 67.5863 SD2= 45.8322 SD3= 49.1891
SE1= 18.7451 SE2= 12.7116 SE3= 13.6426

I1	I2	I3	D1	D2
120	71	49	36	41
195	171	24	52	53
265	194	71	72	70
272	215	57	98	93
256	218	38	130	120
290	237	53	168	149
291	258	33	202	182
311	293	18	237	220
290	241	49	280	254
298	241	57	317	292
292	257	35	362	329
263	245	18	402	367
274	251	23	439	396
319	216	103	467	422

RABBIT 13B N= 14

MEAN I1= 266.857 MEAN I2= 222 MEAN DIF= 44.8571
SD1= 51.7893 SD2= 52.7447 SD3= 23.3695
SE1= 13.8413 SE2= 14.0966 SE3= 6.24575

I1	I2	I3	D1	D2
136	184	-48	69	88
172	209	-37	98	125
197	255	-58	126	154
212	254	-42	158	192
209	230	-21	196	226
188	221	-33	247	262
189	203	-14	272	300
164	192	-28	308	328
201	207	-6	362	366
176	196	-20	404	400
206	218	-12	452	436
205	208	-3	497	468
223	219	4	528	500

RABBIT 15A N= 13

MEAN I1= 190.615 MEAN I2= 215.077 MEAN DIF= -24.4615
SD1= 23.6239 SD2= 21.5076 SD3= 18.568
SE1= 6.5521 SE2= 5.96514 SE3= 5.14983

I1	I2	I3	D1	D2
152	176	-24	52	104
167	190	-23	83	138
194	230	-36	112	171
193	223	-30	136	204
201	211	-10	170	238
283	202	81	210	277
282	191	91	244	317
174	162	12	276	355
188	195	-7	320	394
173	172	1	364	432
194	179	15	408	468
191	191	0	452	498
197	190	7	495	513
205	166	39	536	534

RABBIT 15B N= 14

MEAN I1= 199.571 MEAN I2= 191.286 MEAN DIF= 8.28571
SD1= 38.0176 SD2= 20.1511 SD3= 38.5016
SE1= 10.1606 SE2= 5.3856 SE3= 10.29

MANDIBULAR MIDDLE THIRD
STAINED, NO BLEACH

I1	I2	I3	D1	D2
199	181	18	68	66
199	218	-19	92	87
220	248	-28	120	116
272	310	-38	149	149
248	287	-39	186	184
250	260	-10	220	220
258	260	-2	256	254
264	255	9	287	280
250	241	9	315	310
238	225	13	350	344
203	202	1	384	375
218	192	26	411	403
218	207	11	440	428
203	206	-3	460	452
228	203	25	479	472

RABBIT 10A N= 15

MEAN I1= 231.2 MEAN I2= 233 MEAN DIF=-1.8
SD1= 24.8458 SD2= 36.8549 SD3= 21.1329
SE1= 6.41516 SE2= 9.5159 SE3= 5.45649

I1	I2	I3	D1	D2
189	182	7	78	81
224	211	13	108	116
245	248	-3	142	145
265	259	6	174	175
264	257	7	206	212
283	240	43	252	248
271	222	49	284	281
263	226	37	314	312
263	235	28	346	342
253	220	33	378	374
209	217	-8	413	396
219	200	19	433	424
210	192	18	462	443

RABBIT 10B N= 13

MEAN I1= 242.923 MEAN I2= 223.769 MEAN DIF=
19.1538
SD1= 29.3129 SD2= 23.931 SD3= 17.7569
SE1= 8.12992 SE2= 6.63726 SE3= 4.92488

I1	I2	I3	D1	D2
250	191	59	49	39
250	191	59	68	56
302	236	66	86	79
316	260	56	113	103
310	255	55	143	127
368	304	64	172	156
275	295	-20	203	184
383	309	74	245	217
366	311	55	278	252
371	307	64	311	289
385	305	80	348	322
333	278	55	388	357
313	302	11	418	389

RABBIT 13A N= 13

MEAN I1= 324.769 MEAN I2= 272.615 MEAN DIF= 52.1538
SD1= 47.7862 SD2= 43.3389 SD3= 27.015
SE1= 13.2535 SE2= 12.02 SE3= 7.4926

I1	I2	I3	D1	D2
162	215	-53	37	43
264	215	49	51	62
253	246	7	84	84
262	262	0	93	106
301	245	56	123	133
312	281	31	148	164
344	273	71	180	196
336	271	65	217	230
343	270	73	252	270
330	267	63	296	308
332	271	61	334	344
332	228	104	372	377
298	230	68	409	412

RABBIT 13B N= 13

MEAN I1= 297.615 MEAN I2= 251.846 MEAN DIF= 45.7692
SD1= 51.718 SD2= 23.3375 SD3= 40.6697
SE1= 14.344 SE2= 6.47267 SE3= 11.2797

I1	I2	I3	D1	D2
163	153	10	42	59
200	190	10	62	84
191	216	-25	82	109
211	224	-13	106	131
200	236	-36	134	161
202	241	-39	161	191
237	243	-6	198	224
202	206	-4	232	257
229	247	-18	268	293
235	233	2	309	333
269	271	-2	348	367
283	261	22	390	405
311	262	49	434	438
279	307	-28	478	474
257	291	-34	516	509
272	253	19	546	535

RABBIT 15A N= 16

MEAN I1= 233.813 MEAN I2= 239.625 MEAN DIF= -5.8125
SD1= 41.1229 SD2= 37.7039 SD3= 24.2768
SE1= 10.2807 SE2= 9.42597 SE3= 6.0692

I1	I2	I3	D1	D2
157	164	-7	48	87
149	167	-18	72	113
167	207	-40	99	141
204	223	-19	125	174
205	215	-10	152	214
196	212	-16	184	249
204	193	11	220	285
206	184	22	258	320
194	190	4	294	354
221	186	35	330	395
174	194	-20	369	426
212	185	27	410	456
213	211	2	444	487
225	201	24	475	511
246	258	-12	502	528

RABBIT 15B N= 15

MEAN I1= 198.2 MEAN I2= 199.333 MEAN DIF= -1.13333
SD1= 26.4662 SD2= 23.4114 SD3= 21.3303
SE1= 6.83353 SE2= 6.04481 SE3= 5.50746

MANDIBULAR GINGIVAL THIRD
STAINED, NO BLEACH

I1	I2	I3	D1	D2
100	60	40	38	35
160	142	18	57	46
174	157	17	73	61
210	164	46	98	84
217	192	25	127	113
257	224	33	163	142
227	245	-18	192	170
263	231	32	224	204
255	233	22	258	238
237	197	40	292	270
215	204	11	324	300
220	218	2	354	330
215	199	16	381	358
215	210	5	403	389
215	185	30	422	416
181	179	2	434	448
220	180	40	448	461

RABBIT 10A N= 17

MEAN I1= 210.647 MEAN I2= 189.412 MEAN DIF= 21.2353
SD1= 39.6389 SD2= 43.6134 SD3= 17.1739
SE1= 9.61385 SE2= 10.5778 SE3= 4.16527

I1	I2	I3	D1	D2
152	149	3	40	36
210	168	42	52	60
222	203	19	77	82
246	231	15	101	104
240	241	-1	131	133
287	253	34	160	165
257	261	-4	229	225
238	231	7	260	259
258	218	40	292	288
285	201	84	321	315
245	197	48	348	340
260	211	49	374	366
241	197	44	399	389
250	222	28	414	416
260	204	56	434	438
231	201	30	451	456

RABBIT 10B N= 16

MEAN I1= 242.625 MEAN I2= 211.75 MEAN DIF= 30.875
SD1= 31.36 SD2= 28.8941 SD3= 23.7287
SE1= 7.84 SE2= 7.22351 SE3= 5.93217

I1	I2	I3	D1	D2
147	155	-8	48	44
200	239	-39	64	56
232	250	-18	82	75
255	287	-32	108	99
293	282	11	132	124
279	317	-38	161	152
306	300	6	190	181
325	350	-25	228	211
332	334	-2	256	250
362	346	16	290	287
334	372	-38	326	324

RABBIT 13A N= 11

MEAN I1= 278.636 MEAN I2= 293.818 MEAN DIF= -15.1818
SD1= 64.9712 SD2= 62.0996 SD3= 20.803
SE1= 19.5895 SE2= 18.7237 SE3= 6.27233

I1	I2	I3	D1	D2
211	84	127	44	43
208	193	15	62	54
248	206	42	86	74
252	233	19	112	95
298	265	33	141	123
285	249	36	168	148
322	292	30	200	179
320	301	19	233	213
339	299	40	269	246
348	333	15	303	278
304	306	-2	339	312

RABBIT 13B N= 11

MEAN I1= 285 MEAN I2= 251 MEAN DIF= 34
SD1= 48.859 SD2= 70.7361 SD3= 33.5231
SE1= 14.7315 SE2= 21.3277 SE3= 10.1076

I1	I2	I3	D1	D2
158	185	-27	36	36
177	200	-23	544	54
182	210	-28	72	72
190	213	-23	92	93
228	263	-35	114	120
219	233	-14	146	150
223	280	-57	180	182
226	240	-14	214	214
262	281	-19	250	249
279	304	-25	289	283
279	308	-29	324	316
253	312	-59	360	354
252	275	-23	399	390
244	292	-48	436	428
239	298	-59	470	460
263	290	-27	506	492

RABBIT 15A N= 16

MEAN I1= 229.625 MEAN I2= 261.5 MEAN DIF= -31.875
SD1= 36.7784 SD2= 41.9635 SD3= 15.3444
SE1= 9.1946 SE2= 10.4909 SE3= 3.8361

I1	I2	I3	D1	D2
241	168	73	42	36
262	165	97	52	58
268	181	87	68	78
284	181	103	88	98
274	189	85	112	124
306	211	95	138	151
294	200	94	170	184
311	219	92	204	220
327	220	107	234	253
316	252	64	274	283
298	261	37	308	318
327	291	36	340	351
332	297	35	380	386
284	293	-9	421	425
329	298	31	456	466

RABBIT 15B N= 15

MEAN I1= 296.867 MEAN I2= 228.4 MEAN DIF= 68.4667
SD1= 27.7305 SD2= 49.4568 SD3= 34.463
SE1= 7.15999 SE2= 12.7697 SE3= 8.8983

MAXILLARY INCISAL THIRD
STAINED, 1 BLEACH

I1	I2	I3	D1	D2
126	148	-22	286	296
146	143	3	321	326
186	148	38	360	354
166	153	13	392	382
151	154	-3	422	406
141	126	15	454	438

RABBIT 6 N= 6

MEAN I1= 152.667 MEAN I2= 145.333 MEAN DIF= 7.33333
SD1= 20.8966 SD2= 10.2697 SD3= 20.0865
SE1= 8.53099 SE2= 4.19259 SE3= 8.20027

I1	I2	I3	D1	D2
374	286	88	168	224
352	236	116	220	270
354	259	95	264	304
394	272	122	312	340
372	254	118	357	378
342	222	120	384	410
347	247	100	394	429
314	244	70	408	440

RABBIT 8 N= 8

MEAN I1= 356.125 MEAN I2= 252.5 MEAN DIF= 103.625
SD1= 24.1569 SD2= 20.1849 SD3= 18.6236
SE1= 8.54074 SE2= 7.13643 SE3= 6.58444

I1	I2	I3	D1	D2
354	291	63	166	104
309	284	25	199	148
334	326	8	230	174
330	314	16	254	201
318	280	38	278	226
267	238	29	304	253
280	278	2	334	281
277	265	12	354	304
240	233	7	376	325
265	239	26	392	340

RABBIT 9 N= 10

MEAN I1= 297.4 MEAN I2= 274.8 MEAN DIF= 22.6
SD1= 36.746 SD2= 31.6607 SD3= 18.1488
SE1= 11.6201 SE2= 10.012 SE3= 5.73914

I1	I2	I3	D1	D2
318	235	83	208	89
344	241	103	239	118
375	310	65	268	153
373	336	37	288	176
356	342	14	317	205
356	329	27	344	241
304	329	-25	365	274
316	353	-37	382	300

RABBIT 11 N= 8

MEAN I1= 342.75 MEAN I2= 309.375 MEAN DIF= 33.375
SD1= 27.0911 SD2= 45.7538 SD3= 49.4251
SE1= 9.57816 SE2= 16.1764 SE3= 17.4744

MAXILLARY MIDDLE THIRD
STAINED, 1 BLEACH

I1	I2	I3	D1	D2
166	122	44	232	231
178	130	48	268	264
180	122	58	300	292
170	143	27	330	324
181	171	10	362	352
190	153	37	392	380
164	140	24	419	406
189	144	45	436	412
173	126	47	456	422
145	146	-1	476	434

RABBIT 6 N= 10

MEAN I1= 173.6 MEAN I2= 139.7 MEAN DIF= 33.9
SD1= 13.3433 SD2= 15.3988 SD3= 18.6217
SE1= 4.21953 SE2= 4.869 52 SE3= 5.88869

I1	I2	I3	D1	D2
366	276	90	92	174
334	294	40	134	217
366	311	55	176	249
377	309	68	210	284
375	305	70	247	324
395	279	116	287	360
383	252	131	320	389
408	199	209	352	414
360	225	135	379	432

RABBIT 8 N= 9

MEAN I1= 373.778 MEAN I2= 272.222 MEAN DIF= 101.556
SD1= 21.2472 SD2= 39.474 SD3= 52.3811
SE1= 7.08241 SE2= 13.158 SE3= 17.4604

I1	I2	I3	D1	D2
335	262	73	99	61
320	278	42	132	88
361	307	54	164	116
361	302	59	191	144
342	304	38	221	174
304	293	11	252	204
307	310	-3	274	231
317	324	-7	300	252
287	318	-31	322	278
276	322	-46	340	300
213	268	-55	358	324
229	264	-35	368	347
302	250	52	382	363
272	237	35	392	374

RABBIT 9 N= 14

MEAN I1= 301.857 MEAN I2= 288.5 MEAN DIF= 13.3571

SD1= 44.0975 SD2= 28.3759 SD3= 42.8515

SE1= 11.7856 SE2= 7.58378 SE3= 11.4525

I1	I2	I3	D1	D2
343	169	174	156	9
362	253	109	190	29
396	278	118	218	54
423	316	107	244	80
414	321	93	270	116
404	330	74	296	149
378	316	62	321	186
384	397	-13	347	220
308	339	-31	368	246
285	397	-112	382	278

RABBIT 11 N= 10

MEAN I1= 369.7 MEAN I2= 311.6 MEAN DIF= 58.1

SD1= 45.5803 SD2= 67.2478 SD3= 85.2558

SE1= 14.4138 SE2= 21.2656 SE3= 26.9602

MAXILLARY GINGIVAL THIRD
STAINED, 1 BLEACH

I1	I2	I3	D1	D2
195	178	17	118	178
207	179	28	154	214
212	199	13	187	238
218	210	8	212	270
229	221	8	242	294
199	208	-9	270	322
211	204	7	300	348
240	224	16	326	372
226	212	14	354	398
216	213	3	380	423
212	198	14	406	439
232	212	20	428	448

RABBIT 6 N= 12

MEAN I1= 216.417 MEAN I2= 204.833 MEAN DIF= 11.5833
SD1= 13.3856 SD2= 14.4715 SD3= 9.27811
SE1= 3.86409 SE2= 4.17756 SE3= 2.67836

I1	I2	I3	D1	D2
296	258	38	24	104
308	264	44	56	140
375	305	70	247	324
348	282	66	87	174
381	307	74	121	209
399	318	81	157	246
390	325	65	193	286
365	315	50	229	322
408	336	72	256	352
401	319	82	291	381
396	275	121	322	408
358	256	102	353	430
346	248	98	380	445

RABBIT 8 N= 13

MEAN I1= 367 MEAN I2= 292.923 MEAN DIF= 74.0769
SD1= 35.2373 SD2= 30.1426 SD3= 23.4857
SE1= 9.77307 SE2= 8.36006 SE3= 6.51375

I1	I2	I3	D1	D2
167	154	13	72	33
173	164	9	100	66
190	177	13	126	91
192	180	12	122	122
195	174	21	183	156
166	162	4	210	186
177	157	20	234	212
183	164	19	260	234
171	160	11	284	262
172	147	25	308	290
151	133	18	336	312
140	132	8	357	336
154	114	40	376	352
194	114	80	398	368

RABBIT 9 N= 14

MEAN I1= 173.214 MEAN I2= 152.286 MEAN DIF= 20.9286

SD1= 16.9032 SD2= 21.5672 SD3= 19.1812

SE1= 4.51758 SE2= 5.76407 SE3= 5.12639

I1	I2	I3	D1	D2
378	247	131	116	153
386	285	101	150	189
442	342	100	174	226
445	361	84	201	253
423	349	74	228	283
414	333	81	257	319
405	321	84	285	344
473	387	86	320	367
373	270	103	348	386

RABBIT 11 N= 9

MEAN I1= 415.444 MEAN I2= 321.667 MEAN DIF= 93.7778

SD1= 33.7828 SD2= 45.681 SD3= 17.203

SE1= 11.2609 SE2= 15.227 SE3= 5.73435

MANDIBULAR INCISAL THIRD
STAINED, 1 BLEACH

I1	I2	I3	D1	D2
85	53	32	44	39
114	108	6	68	55
140	115	25	89	78
136	131	5	116	103
175	161	14	143	128
167	154	13	172	162
185	156	29	208	191
161	164	-3	244	224
175	155	20	277	259
175	153	22	308	292
157	153	4	338	338
171	152	19	368	354
151	138	13	394	384
143	136	7	421	410
144	140	4	446	434
141	127	14	464	456

RABBIT 6 N= 16

MEAN I1= 151.25 MEAN I2= 137.25 MEAN DIF= 14
SD1= 25.7669 SD2= 27.7405 SD3= 9.93311
SE1= 6.44173 SE2= 6.93512 SE3= 2.48328

I1	I2	I3	D1	D2
73	47	26	45	45
87	46	41	62	72
94	67	27	87	104
111	71	40	114	132
116	78	38	141	162
113	71	42	180	206
131	84	47	220	240
115	90	25	262	274
122	76	46	304	318
128	78	50	350	361
148	75	73	398	402
141	65	76	442	434
122	55	67	474	454

RABBIT 8 N= 13

MEAN I1= 115.462 MEAN I2= 69.4615 MEAN DIF= 46
SD1= 21.0064 SD2= 13.3767 SD3= 16.9066
SE1= 5.82613 SE2= 3.71003 SE3= 4.68905

I1	I2	I3	D1	D2
155	110	45	42	36
210	214	-4	58	52
237	212	25	82	74
295	259	36	110	100
279	312	-33	143	128
321	335	-14	182	162
300	345	-45	222	196
301	309	-8	258	230
301	310	-9	306	266
279	319	-40	352	308
245	285	-40	401	308
255	308	-53	438	381

RABBIT 9 N= 12

MEAN I1= 264.833 MEAN I2= 276.5 MEAN DIF= -11.6667

SD1= 47.3418 SD2= 67.769 SD3= 32.7423

SE1= 13.6664 SE2= 19.5632 SE3= 9.4519

I1	I2	I3	D1	D2
234	140	94	106	147
249	170	79	136	182
310	214	96	162	213
306	210	96	194	247
295	190	105	227	285
267	221	46	259	315
257	194	63	289	349
328	206	122	314	369
244	169	75	342	394
265	212	53	363	416

RABBIT 11 N= 10

MEAN I1= 275.5 MEAN I2= 192.6 MEAN DIF= 82.9

SD1= 31.9696 SD2= 25.7475 SD3= 24.0668

SE1= 10.1097 SE2= 8.14207 SE3= 7.61059

MANDIBULAR MIDDLE THIRD
STAINED, 1 BLEACH

I1	I2	I3	D1	D2
48	33	15	43	38
64	83	-19	66	49
79	74	5	86	74
71	103	-32	118	102
100	115	-15	148	130
101	136	-35	184	154
124	151	-27	215	190
132	154	-22	250	228
123	141	-18	281	266
108	145	-37	313	300
124	142	-18	342	331
90	142	-52	371	363
144	124	20	395	394
100	100	0	416	428
96	130	-34	434	450

RABBIT 6 N= 15

MEAN I1= 100.267 MEAN I2= 118.2 MEAN DIF= -17.9333
SD1= 26.8633 SD2= 34.0613 SD3= 20.2677
SE1= 6.93608 SE2= 8.79459 SE3= 5.23311

I1	I2	I3	D1	D2
23	62	-39	44	39
113	54	59	55	55
105	71	34	73	73
132	72	60	94	102
158	92	66	126	133
163	109	54	157	167
174	110	64	193	196
172	123	49	234	232
169	106	63	270	272
186	88	98	311	308
209	99	110	352	344
205	102	103	394	384
179	100	79	432	416
151	88	63	464	448
117	93	24	484	468

RABBIT 8 N= 15

MEAN I1= 150.4 MEAN I2= 91.2667 MEAN DIF= 59.1333
SD1= 47.2316 SD2= 19.2594 SD3= 35.9997
SE1= 12.1952 SE2= 4.97275 SE3= 9.29509

I1	I2	I3	D1	D2
235	136	99	57	38
253	179	74	81	60
305	223	82	111	82
321	297	24	142	106
323	336	-13	176	134
361	364	-3	218	165
340	350	-10	262	201
365	347	18	314	233
383	337	46	369	270
386	332	54	413	305
367	338	29	448	346

RABBIT 9 N= 11

MEAN I1= 330.818 MEAN I2= 294.455 MEAN DIF= 36.3636

SD1= 50.3007 SD2= 78.1503 SD3= 38.0875

SE1= 15.1662 SE2= 23.5632 SE3= 11.4838

I1	I2	I3	D1	D2
224	171	53	52	101
276	187	89	72	130
334	237	97	93	157
367	287	80	118	188
386	261	125	144	218
393	267	126	174	255
364	274	90	208	286
416	334	82	240	309
333	271	62	270	339
402	305	97	296	366
432	315	117	320	383
334	282	52	346	402
388	310	78	368	416

RABBIT 11 N= 13

MEAN I1= 357.615 MEAN I2= 269.308 MEAN DIF= 88.3077

SD1= 57.9203 SD2= 47.6609 SD3= 24.5234

SE1= 16.0642 SE2= 13.2188 SE3= 6.80157

MANDIBULAR GINGIVAL THIRD
STAINED, 1 BLEACH

I1	I2	I3	D1	D2
60	30	30	38	45
105	53	52	51	64
108	58	50	76	84
130	66	64	96	112
131	100	31	126	150
155	93	62	158	186
151	128	23	187	223
142	108	34	220	259
140	103	37	255	295
145	94	51	284	326
146	99	47	320	364
151	93	58	350	394
117	100	17	378	422

RABBIT 6 N= 13

MEAN I1= 129.308 MEAN I2= 86.5385 MEAN DIF= 42.7692
SD1= 26.465 SD2= 26.872 SD3= 15.1171
SE1= 7.34008 SE2= 7.45294 SE3= 4.19272

I1	I2	I3	D1	D2
180	183	-3	32	60
245	183	62	45	84
268	209	59	62	108
292	239	53	88	138
329	252	77	117	168
356	280	76	150	203
374	275	99	184	242
405	273	132	222	278
418	292	126	258	314
465	292	173	295	348
475	312	163	338	379
469	313	156	380	407
445	306	139	418	440
372	289	83	448	466

RABBIT 8 N= 14

MEAN I1= 363.786 MEAN I2= 264.143 MEAN DIF= 99.6429
SD1= 91.3886 SD2= 44.7521 SD3= 50.3764
SE1= 24.4246 SE2= 11.9605 SE3= 13.4637

I1	I2	I3	D1	D2
224	37	187	38	28
276	119	157	62	42
292	134	158	83	62
335	227	108	115	86
362	255	107	147	112
363	275	88	184	142
354	305	49	224	172
345	323	22	268	206
363	303	60	306	242
335	304	31	354	274
321	307	14	402	316
321	280	41	438	352

RABBIT 9 N= 12

MEAN I1= 324.25 MEAN I2= 239.083 MEAN DIF= 85.1667

SD1= 42.0111 SD2= 92.4234 SD3= 58.5597

SE1= 12.1276 SE2= 26.6803 SE3= 16.9047

I1	I2	I3	D1	D2
206	227	-21	32	44
264	286	-22	48	60
310	343	-33	63	78
308	369	-61	83	102
334	363	-29	108	128
358	422	-64	133	158
373	499	-126	158	189
436	470	-34	188	218
340	357	-17	220	247
361	401	-40	244	274
347	421	-74	273	300
313	359	-46	300	327
298	340	-42	321	355
288	344	-56	346	380
277	379	-102	369	399
284	324	-40	387	416
291	316	-25	399	426

RABBIT 11 N= 17

MEAN I1= 316.941 MEAN I2= 365.882 MEAN DIF= -48.9412

SD1= 51.5236 SD2= 65.1353 SD3= 29.5751

SE1= 12.4963 SE2= 15.7976 SE3= 7.173

MAXILLARY INCISAL THIRD
STAINED 2 BLEACHES

I1	I2	I3	D1	D2
327	146	181	281	268
329	146	183	320	302
353	160	193	353	353
342	156	186	392	368
343	147	196	424	398
297	139	158	444	412

RABBIT 7 N= 6

MEAN I1= 331.833 MEAN I2= 149 MEAN DIF= 182.833
SD1= 19.6002 SD2= 7.64199 SD3= 13.4672
SE1= 8.00174 SE2= 3.11983 SE3= 5.49798

I1	I2	I3	D1	D2
225	88	137	85	97
217	95	122	119	132
219	109	110	152	160
208	109	99	182	188
242	135	107	209	215
248	156	92	234	248
270	172	98	239	273
280	188	92	281	303
257	188	69	311	329
257	166	91	334	352
284	195	89	361	379
260	165	95	382	404
235	165	70	402	426

RABBIT 12 N= 13

MEAN I1= 246.308 MEAN I2= 148.538 MEAN DIF= 97.7692
SD1= 24.5098 SD2= 37.1565 SD3= 18.6152
SE1= 6.7978 SE2= 10.3053 SE3= 5.16293

I1	I2	I3	D1	D2
357	222	135	267	234
357	232	125	302	272
332	244	88	336	308
327	231	96	372	342
269	169	100	408	372
273	172	101	432	398

RABBIT 16 N= 6

MEAN I1= 319.167 MEAN I2= 211.667 MEAN DIF= 107.5

SD1= 39.3366 SD2= 32.6599 SD3= 18.2948
SE1= 16.0591 SE2= 13.3333 SE3= 7.46882

I1	I2	I3	D1	D2
462	327	135	178	160
312	408	-96	198	223
401	326	75	250	228
415	323	92	284	260
404	361	43	314	294
376	311	65	348	326
356	269	87	377	357
317	306	11	394	376

RABBIT 17 N= 8

MEAN I1= 380.375 MEAN I2= 328.875 MEAN DIF= 51.5

SD1= 50.9199 SD2= 40.9998 SD3= 69.7711
SE1= 18.0029 SE2= 14.4956 SE3= 24.6678

I1	I2	I3	D1	D2
267	120	147	103	94
266	119	147	149	140
230	228	2	183	185
230	113	117	185	183
242	150	92	220	224
248	152	96	258	263
275	169	106	298	303
289	183	106	334	339
107	80	27	372	389

RABBIT 18 N= 9

MEAN I1= 239.333 MEAN I2= 146 MEAN DIF= 93.3333

SD1= 53.6004 SD2= 44.0511 SD3= 49.2341
SE1= 17.8668 SE2= 14.6837 SE3= 16.4114

I1	I2	I3	D1	D2
173	37	136	42	20
187	91	96	62	47
174	121	53	86	72
175	124	51	114	100
189	138	51	143	136
203	167	36	178	167
324	252	72	202	204
222	185	37	231	233
212	174	38	268	271
232	175	57	292	303
203	165	38	318	331
182	166	16	346	358
195	183	12	374	380
187	90	97	394	403

RABBIT 19 N= 14

MEAN I1= 204.143 MEAN I2= 147.714 MEAN DIF= 56.4286

SD1= 38.8723 SD2= 52.7161 SD3= 33.8997

SE1= 10.3891 SE2= 14.089 SE3= 9.06006

I1	I2	I3	D1	D2
194	57	137	88	146
200	41	159	120	177
211	48	163	146	200
162	32	130	171	231
181	42	139	201	262
184	20	164	235	294
164	9	155	264	320
173	8	165	288	348
146	2	144	318	372
113	6	107	349	394
103	6	97	375	420
106	12	94	410	434
112	12	100	436	452

RABBIT 20 N= 13

MEAN I1= 157.615 MEAN I2= 22.6923 MEAN DIF= 134.923

SD1= 38.0384 SD2= 18.79 SD3= 27.0199

SE1= 10.55 SE2= 5.21141 SE3= 7.49398

MAXILLARY MIDDLE THIRD
STAINED, 2 BLEACHES

I1	I2	I3	D1	D2
356	172	184	217	172
346	172	174	254	202
386	227	159	288	232
397	252	145	320	262
399	297	102	351	292
378	310	68	382	340
331	323	8	410	362
331	327	4	410	389

RABBIT 7 N= 8

MEAN I1= 365.5 MEAN I2= 260 MEAN DIF= 105.5
SD1= 28.1374 SD2= 64.3295 SD3= 72.214
SE1= 9.94808 SE2= 22.7439 SE3= 25.5315

I1	I2	I3	D1	D2
233	103	130	72	52
257	94	163	104	83
260	100	160	134	110
254	126	128	160	134
260	139	121	186	162
294	153	141	210	192
310	180	130	237	222
317	197	120	260	248
307	212	95	287	269
306	219	87	312	292
320	242	78	336	315
300	247	53	364	345
267	237	30	388	372
257	213	44	400	400

RABBIT 12 N= 14

MEAN I1= 281.571 MEAN I2= 175.857 MEAN DIF=
105.714
SD1= 28.7528 SD2= 55.6885 SD3= 42.1908
SE1= 7.68452 SE2= 14.8834 SE3= 11.276

I1	I2	I3	D1	D2
375	275	100	182	164
393	294	99	210	196
408	290	118	242	228
430	296	134	274	255
385	270	115	308	289
403	289	114	343	328
366	255	111	377	362
366	278	88	400	391
312	219	93	426	413

RABBIT 16 N= 9

MEAN I1= 382 MEAN I2= 274 MEAN DIF= 108
SD1= 33.5634 SD2= 24.454 SD3= 14.3178
SE1= 11.1878 SE2= 8.15135 SE3= 4.77261

I1	I2	I3	D1	D2
413	302	111	76	118
432	318	114	114	156
482	344	138	152	190
475	341	134	185	217
476	345	131	218	247
446	328	118	244	278
470	325	145	276	308
468	327	141	302	333
384	223	161	334	360
397	237	160	364	378
340	263	77	384	390

RABBIT 17 N= 11

MEAN I1= 434.818 MEAN I2= 304.818 MEAN DIF= 130
SD1= 46.3548 SD2= 43.726 SD3= 24.2033
SE1= 13.9765 SE2= 13.1839 SE3= 7.29757

I1	I2	I3	D1	D2
227	88	139	57	42
239	107	132	90	74
233	113	120	126	108
274	144	130	163	144
310	158	152	198	182
276	176	100	238	227
320	165	155	272	260
118	58	60	304	292
279	177	102	336	326
278	212	66	362	353
238	214	24	381	382
231	180	51	393	404

RABBIT 18 N= 12

MEAN I1= 251.917 MEAN I2= 149.333 MEAN DIF= 102.583

SD1= 52.3979 SD2= 48.7019 SD3= 43.0971

SE1= 15.126 SE2= 14.059 SE3= 12.441

I1	I2	I3	D1	D2
201	95	106	33	36
223	130	93	57	58
228	155	73	78	83
228	176	52	104	110
254	238	16	135	138
353	181	172	162	168
243	184	59	193	195
239	210	29	225	226
253	210	43	252	251
253	178	75	279	274
218	201	17	300	294
223	192	31	321	318
195	214	-19	344	338
204	210	-6	362	360

RABBIT 19 N= 14

MEAN I1= 236.786 MEAN I2= 183.857 MEAN DIF= 52.9286

SD1= 38.5909 SD2= 37.1936 SD3= 49.5991

SE1= 10.3139 SE2= 9.9404 SE3= 13.2559

I1	I2	I3	D1	D2
192	76	116	40	100
224	151	73	62	132
199	88	111	83	160
230	70	160	107	181
221	88	133	136	200
233	67	166	164	220
217	56	161	189	262
222	60	162	214	273
196	36	160	243	299
186	27	159	270	324
187	34	153	294	353
172	44	128	316	380
165	-4	169	334	406
152	47	105	348	428
158	34	124	368	448

RABBIT 20 N= 15

MEAN I1= 196.933 MEAN I2= 58.2667 MEAN DIF= 138.667

SD1= 26.9403 SD2= 35.62 SD3= 28.4421

SE1= 6.95596 SE2= 9.19703 SE3= 7.34372

MAXILLARY GINGIVAL THIRD
STAINED, 2 BLEACHES

I1	I2	I3	D1	D2
325	149	176	143	126
339	174	165	174	160
342	192	150	203	188
354	209	145	234	218
364	234	130	264	247
360	261	99	295	282
329	266	63	332	312
365	299	66	358	346
356	294	62	384	370
371	303	68	407	404
307	269	38	416	424
321	249	72	425	446

RABBIT 7 N= 12

MEAN I1= 344.417 MEAN I2= 241.583 MEAN DIF= 102.833
SD1= 20.407 SD2= 50.7336 SD3= 47.627
SE1= 5.891 SE2= 14.6455 SE3= 13.7487

I1	I2	I3	D1	D2
274	112	162	23	23
300	124	176	54	54
308	147	161	79	80
300	149	151	104	108
313	168	145	135	140
325	178	147	160	168
375	210	165	186	197
366	222	144	208	223
366	232	134	234	250
360	247	113	261	278
398	283	115	289	307
363	275	88	317	332
339	278	61	342	356
337	273	64	369	378
344	276	68	395	396
271	257	14	412	414
225	218	7	425	429

RABBIT 12 N= 17

MEAN I1= 327.294 MEAN I2= 214.647 MEAN DIF= 112.647
SD1= 44.4912 SD2= 58.0323 SD3= 53.1495
SE1= 10.7907 SE2= 14.0749 SE3= 12.8906

I1	I2	I3	D1	D2
359	280	79	96	103
403	289	114	122	129
397	280	117	149	156
397	297	100	176	187
408	286	122	210	217
392	283	109	248	251
381	306	75	284	283
450	364	86	314	314
412	311	101	349	348
393	328	65	379	386
366	262	104	412	420
312	233	79	438	440
324	237	87	456	456

RABBIT 16 N= 13

MEAN I1= 384.154 MEAN I2= 288.923 MEAN DIF= 95.2308
SD1= 36.8575 SD2= 35.0701 SD3= 17.9544
SE1= 10.2224 SE2= 9.72669 SE3= 4.97964

I1	I2	I3	D1	D2
228	207	21	19	44
343	245	98	44	80
416	317	99	74	114
417	329	88	104	148
445	357	88	140	183
415	339	76	179	216
438	344	94	218	246
449	383	66	245	275
428	344	84	280	308
483	404	79	314	342
446	331	115	344	370
420	301	119	369	388
389	359	30	390	400

RABBIT 17 N= 13

MEAN I1= 409 MEAN I2= 327.692 MEAN DIF= 81.3077
SD1= 63.7247 SD2= 52.9345 SD3= 28.7848
SE1= 17.674 SE2= 14.6814 SE3= 7.98346

I1	I2	I3	D1	D2
206	110	96	19	37
273	152	121	49	64
299	173	126	77	97
338	193	145	108	140
328	232	96	145	173
341	72	269	180	210
297	197	100	214	248
137	215	-78	246	280
349	225	124	284	308
371	220	151	314	336
361	203	158	345	362
325	163	162	368	381
277	167	110	384	393
274	163	111	396	400

RABBIT 18 N= 14

MEAN I1= 298.286 MEAN I2= 177.5 MEAN DIF= 120.786

SD1= 63.8537 SD2= 45.2323 SD3= 72.3115

SE1= 17.0656 SE2= 12.0888 SE3= 19.3261

I1	I2	I3	D1	D2
175	150	25	22	42
230	210	20	44	64
263	313	-50	67	89
373	231	142	88	114
284	222	62	112	148
268	270	-2	148	177
296	278	18	176	205
312	239	73	200	234
281	269	12	228	260
307	234	73	254	286
259	276	-17	283	308
303	240	63	311	329
305	245	60	337	352
287	249	38	361	373
315	202	113	378	388

RABBIT 19 N= 15

MEAN I1= 283.867 MEAN I2= 241.867 MEAN DIF= 42

SD1= 44.1408 SD2= 38.4984 SD3= 49.4787

SE1= 11.3971 SE2= 9.94023 SE3= 12.7753

I1	I2	I3	D1	D2
82	96	-14	20	57
172	82	90	45	82
168	112	56	68	104
158	82	76	84	122
177	115	62	108	150
169	116	53	137	172
167	115	52	168	190
165	112	53	193	207
183	87	96	220	230
207	65	142	246	254
170	45	125	278	282
158	60	98	306	304
170	53	117	332	334
133	36	97	360	364
127	21	106	384	392

RABBIT 20 N= 15

MEAN I1= 160.4 MEAN I2= 79.8 MEAN DIF= 80.6
SD1= 28.6576 SD2= 31.7382 SD3= 38.6112
SE1= 7.39936 SE2= 8.19477 SE3= 9.96938

MANDIBULAR INCISAL THIRD
STAINED, 2 BLEACHES

I1	I2	I3	D1	D2
225	82	143	94	83
275	95	180	122	114
338	143	195	146	143
337	167	170	176	172
350	177	173	206	200
322	195	127	242	234
307	192	115	272	268
308	212	96	304	298
325	225	100	338	328
310	206	104	364	353
295	182	113	406	382
306	200	106	424	404

RABBIT 7 N= 12

MEAN I1= 308.167 MEAN I2= 173 MEAN DIF= 135.167
SD1= 33.2288 SD2= 45.0111 SD3= 35.4884
SE1= 9.59232 SE2= 12.9936 SE3= 10.2446

I1	I2	I3	D1	D2
106	50	56	35	36
176	86	90	52	61
207	132	75	71	86
264	147	117	94	116
286	180	106	124	149
346	215	131	152	184
334	198	136	179	220
346	219	127	210	261
365	220	145	248	305
396	236	160	287	343
346	232	114	325	380
345	173	172	368	417
368	188	180	408	450

RABBIT 12 N= 13

MEAN I1= 298.846 MEAN I2= 175.077 MEAN DIF=
123.769
SD1= 86.9529 SD2= 57.3272 SD3= 36.5517
SE1= 24.1164 SE2= 15.8997 SE3= 10.1376

I1	I2	I3	D1	D2
289	104	185	63	38
360	100	260	80	56
375	119	256	106	82
411	150	261	134	102
418	158	260	166	130
444	197	247	198	163
428	198	230	234	195
473	220	253	268	230
446	219	227	312	264
455	217	238	344	303
428	199	229	380	346
427	187	240	418	380
446	190	256	448	408
442	203	239	474	436
409	195	214	500	468

RABBIT 14 N= 15

MEAN I1= 416.733 MEAN I2= 177.067 MEAN DIF=
239.667
SD1= 45.8685 SD2= 40.9869 SD3= 20.8144
SE1= 11.8432 SE2= 10.5828 SE3= 5.37425

I1	I2	I3	D1	D2
243	37	206	90	36
275	77	198	115	47
295	102	193	142	63
270	123	147	172	86
273	153	120	208	114
298	166	132	245	142
291	239	52	282	168
330	233	97	314	208
295	232	63	352	245

RABBIT 16 N= 9

MEAN I1= 285.556 MEAN I2= 151.333 MEAN DIF=
134.222
SD1= 24.1356 SD2= 73.241 SD3= 57.297
SE1= 8.0452 SE2= 24.4137 SE3= 19.099

I1	I2	I3	D1	D2
220	116	104	40	36
268	173	95	60	53
311	210	101	85	72
338	199	139	110	100
357	263	94	142	130
354	245	109	174	163
351	276	75	208	196
348	326	22	240	228
327	352	-25	278	264
388	361	27	320	303
330	289	41	362	348
313	242	71	398	383
313	219	94	432	422
318	129	189	457	456

RABBIT 17 N= 14

MEAN I1= 324 MEAN I2= 242.857 MEAN DIF= 81.1429
SD1= 41.1657 SD2= 75.4115 SD3= 53.0919
SE1= 11.002 SE2= 20.1546 SE3= 14.1894

I1	I2	I3	D1	D2
49	11	38	41	48
79	23	56	58	64
89	30	59	80	92
97	30	67	106	150
77	54	23	135	184
77	41	36	166	221
77	37	40	202	253
80	45	35	234	290
61	53	8	267	330
67	35	32	300	378
66	49	17	334	416

RABBIT 18 N= 11

MEAN I1= 74.4545 MEAN I2= 37.0909 MEAN DIF=
37.3636
SD1= 13.2164 SD2= 13.2019 SD3= 17.9459
SE1= 3.98489 SE2= 3.98053 SE3= 5.41089

I1	I2	I3	D1	D2
113	33	80	33	62
190	35	155	50	85
213	47	166	64	112
215	82	133	87	144
246	99	147	114	178
291	100	191	146	214
403	124	279	176	254
298	187	111	212	283
274	120	154	254	327
300	119	181	288	365
307	122	185	324	398
292	111	181	363	420
284	125	159	401	430

RABBIT 19 N= 13

MEAN I1= 263.538 MEAN I2= 100.308 MEAN DIF= 163.231

SD1= 70.2586 SD2= 42.8007 SD3= 46.8066

SE1= 19.4862 SE2= 11.8708 SE3= 12.9818

I1	I2	I3	D1	D2
25	64	-39	35	36
42	44	-2	49	54
54	36	18	64	82
92	62	30	88	104
96	67	29	114	132
87	100	-13	143	161
101	100	1	172	194
91	95	-4	198	228
89	93	-4	232	254
90	103	-13	266	291
101	121	-20	292	320
108	104	4	326	362
90	105	-15	360	398
87	84	3	392	431
94	91	3	417	460
73	88	-15	442	476

RABBIT 20 N= 16

MEAN I1= 82.5 MEAN I2= 84.8125 MEAN DIF=-2.3125

SD1= 22.8852 SD2= 23.6875 SD3= 17.8128

SE1= 5.72131 SE2= 5.92187 SE3= 4.4532

MANDIBULAR MIDDLE THIRD
STAINED, 2 BLEACHES

I1	I2	I3	D1	D2
203	151	52	84	54
227	203	24	112	78
287	222	65	140	102
283	278	5	167	124
301	298	3	194	148
292	306	-14	228	178
283	301	-18	263	206
293	315	-22	294	235
321	337	-16	325	268
299	350	-51	352	298
284	337	-53	378	324
289	342	-53	405	354
264	295	-31	422	368

RABBIT 7 N= 13

MEAN I1= 278.923 MEAN I2= 287.308 MEAN DIF= -8.38462
SD1= 31.5422 SD2= 60.1226 SD3= 37.7106
SE1= 8.74823 SE2= 16.675 SE3= 10.459

I1	I2	I3	D1	D2
150	152	-2	35	35
260	196	64	47	50
296	254	42	68	71
375	298	77	88	94
350	310	40	117	123
399	351	48	149	154
447	379	68	178	188
384	345	39	213	223
394	329	65	248	257
417	357	60	285	289
388	358	30	323	324

RABBIT 12 N= 11

MEAN I1= 350.909 MEAN I2= 302.636 MEAN DIF= 48.2727
SD1= 85.1275 SD2= 72.9277 SD3= 22.213
SE1= 25.6669 SE2= 21.9885 SE3= 6.69748

I1	I2	I3	D1	D2
289	77	212	44	40
305	91	214	62	60
296	133	163	83	81
338	126	212	108	106
362	164	198	136	134
406	184	222	163	166
374	237	137	198	198
381	226	155	230	236
409	238	171	262	272
408	239	169	296	309
419	254	165	334	344
408	244	164	362	378
414	231	183	392	406
418	240	178	428	438
405	218	187	458	466
448	232	216	484	490
392	208	184	506	506

RABBIT 14 N= 17

MEAN I1= 380.706 MEAN I2= 196.588 MEAN DIF= 184.118
SD1= 47.2212 SD2= 57.0987 SD3= 24.7787
SE1= 11.4528 SE2= 13.8485 SE3= 6.00972

I1	I2	I3	D1	D2
169	63	106	38	30
188	123	65	52	42
201	145	56	73	58
219	182	37	93	80
201	232	-31	118	107
227	260	-33	148	132
198	267	-69	180	164
267	285	-18	210	194
273	279	-6	248	228
263	308	-45	286	266
282	337	-55	326	298
261	355	-94	360	338
256	299	-43	394	374
308	308	0	428	414
283	318	-35	458	442
237	330	-93	486	469

RABBIT 16 N= 16

MEAN I1= 239.563 MEAN I2= 255.688 MEAN DIF= -16.125
SD1= 40.3716 SD2= 84.7567 SD3= 56.9127
SE1= 10.0929 SE2= 21.1892 SE3= 14.2282

I1	I2	I3	D1	D2
290	94	196	37	38
342	173	169	56	64
434	207	227	79	87
436	234	202	108	114
463	251	212	136	139
458	308	150	166	178
397	325	72	200	206
431	261	170	234	244
389	306	83	266	282
387	255	132	302	322
453	232	221	338	357
432	230	202	382	393
393	234	159	418	441
324	245	79	448	473

RABBIT 17 N= 14

MEAN I1= 402.071 MEAN I2= 239.643 MEAN DIF= 162.429
SD1= 52.7016 SD2= 58.2734 SD3= 53.3172
SE1= 14.0851 SE2= 15.5742 SE3= 14.2496

I1	I2	I3	D1	D2
45	55	-10	38	36
77	84	-7	55	66
13	17	-4	88	94
86	106	-20	113	124
121	114	7	147	156
125	112	13	185	196
102	112	-10	216	227
115	103	12	254	261
115	117	-2	288	294
113	110	3	321	335
131	115	16	366	378
117	117	0	407	420
121	119	2	447	458
107	108	-1	481	488

RABBIT 18 N= 14

MEAN I1= 99.1429 MEAN I2= 99.2143 MEAN DIF= -7.14286E-2
SD1= 33.67 SD2= 29.113 SD3= 10.0036
SE1= 8.99869 SE2= 7.78077 SE3= 2.67357

I1	I2	I3	D1	D2
198	27	171	35	33
268	51	217	53	54
272	67	205	73	73
315	92	223	96	98
315	127	188	121	128
332	127	205	153	161
310	127	183	189	188
321	218	103	228	222
326	135	191	259	256
286	126	160	300	296
287	131	156	342	334
308	114	194	376	369
254	91	163	416	400
287	93	194	444	424

RABBIT 19 N= 14

MEAN I1= 291.357 MEAN I2= 109 MEAN DIF= 182.357
SD1= 35.7031 SD2= 45.6138 SD3= 30.6986
SE1= 9.54205 SE2= 12.1908 SE3= 8.20453

I1	I2	I3	D1	D2
56	20	36	34	34
64	34	30	52	49
78	58	20	72	71
88	58	30	94	93
102	83	19	120	120
101	76	25	148	148
118	99	19	179	175
127	95	32	208	202
123	102	21	234	234
98	113	-15	266	261
96	96	0	300	290
116	94	22	330	332
92	110	-18	366	364
96	86	10	398	400
98	122	-24	426	428

RABBIT 20 N= 15

MEAN I1= 96.8667 MEAN I2= 83.0667 MEAN DIF=
13.8
SD1= 20.1064 SD2= 29.1289 SD3= 19.199
SE1= 5.19145 SE2= 7.52106 SE3= 4.95715

MANDIBULAR GINGIVAL THIRD
STAINED, 2 BLEACHES

I 1	I 2	I 3	D1	D2
158	157	1	42	38
215	229	-14	62	52
236	231	5	87	70
270	273	-3	109	92
284	294	-10	134	114
307	301	6	160	140
308	318	-10	190	165
323	335	-12	220	192
331	351	-20	250	226
357	345	12	281	256
356	324	32	310	284
363	325	38	334	312
341	284	57	360	334
389	348	41	384	358
391	315	76	407	384
357	342	15	428	402
333	305	28	446	424

RABBIT 7 N= 17

MEAN I 1= 312.882 MEAN I 2= 298.647 MEAN DIF= 14.2353
SD1= 63.2444 SD2= 51.8024 SD3= 27.2638
SE1= 15.339 SE2= 12.5639 SE3= 6.61245

I 1	I 2	I 3	D1	D2
217	95	122	35	36
279	153	126	50	50
300	204	96	72	70
359	255	104	98	93
399	257	142	124	118
372	332	40	156	145
361	364	-3	187	178
390	331	59	219	211
387	342	45	248	242
379	341	38	278	276

RABBIT 12 N= 10

MEAN I 1= 344.3 MEAN I 2= 267.4 MEAN DIF= 76.9
SD1= 59.4438 SD2= 91.6651 SD3= 47.5849
SE1= 18.7978 SE2= 28.987 SE3= 15.0477

I1	I2	I3	D1	D2
239	203	36	38	50
273	263	10	57	70
314	306	8	79	85
322	328	-6	104	113
370	371	-1	124	138
374	384	-10	151	172
385	403	-18	182	205
390	385	5	218	236
395	380	15	250	269
401	355	46	284	303
402	328	74	309	342
392	308	84	347	378
399	310	89	383	413
400	293	107	413	447

RABBIT 14 N= 14

MEAN I1= 361.143 MEAN I2= 329.786 MEAN DIF= 31.3571

SD1= 52.9061 SD2= 55.1434 SD3= 41.5315

SE1= 14.1397 SE2= 14.7377 SE3= 11.0998

I1	I2	I3	D1	D2
110	48	62	30	34
251	165	86	45	48
278	190	88	63	64
329	252	77	87	84
313	275	38	110	110
394	291	103	137	133
356	342	14	170	160
344	310	34	207	196
373	321	52	237	228
363	384	-21	272	262
381	402	-21	308	298
407	369	38	346	334
429	399	30	387	370
363	349	14	417	408
385	365	20	447	440
331	350	-19	478	472

RABBIT 16 N= 16

MEAN I1= 337.938 MEAN I2= 300.75 MEAN DIF= 37.1875

SD1= 76.1485 SD2= 96.7068 SD3= 39.2245

SE1= 19.0371 SE2= 24.1767 SE3= 9.80613

I1	I2	I3	D1	D2
257	134	123	36	36
357	232	125	50	48
385	287	98	72	67
425	321	104	96	93
445	365	80	123	118
418	390	28	158	149
449	358	91	192	182
411	412	-1	229	218
385	335	50	266	257
430	346	84	303	293
472	431	41	343	328
469	412	57	379	372
350	381	-31	420	404
317	307	10	452	434
329	276	53	480	462

RABBIT 17 N= 15

MEAN I1= 393.267 MEAN I2= 332.467 MEAN DIF=
60.8
SD1= 61.4346 SD2= 78.1225 SD3= 45.9397
SE1= 15.8623 SE2= 20.1711 SE3= 11.8616

I1	I2	I3	D1	D2
87	132	-45	33	34
143	198	-55	49	48
98	222	-124	67	68
191	272	-81	90	92
207	418	-211	119	114
264	321	-57	148	148
275	290	-15	181	182
245	350	-105	216	218
304	298	6	252	249
231	298	-67	292	280
307	343	-36	334	318
291	263	28	369	352
267	304	-37	401	388
324	273	51	428	420
250	248	2	452	443

RABBIT 18 N= 15

MEAN I1= 232.267 MEAN I2= 282 MEAN DIF=-49.7333
SD1= 73.9464 SD2= 67.758 SD3= 65.1838
SE1= 19.0929 SE2= 17.495 SE3= 16.8304

I1	I2	I3	D1	D2
128	123	5	44	44
157	45	112	65	62
178	337	-159	90	85
185	176	9	120	116
194	206	-12	150	145
197	176	21	184	182
193	158	35	220	215
198	156	42	256	246
208	148	60	288	284
216	167	49	324	320
232	162	70	355	356

RABBIT 19 N= 11

MEAN I1= 189.636 MEAN I2= 168.545 MEAN DIF=
21.0909
SD1= 28.2888 SD2= 69.2566 SD3= 68.9296
SE1= 8.52939 SE2= 20.8816 SE3= 20.7831

I1	I2	I3	D1	D2
19	63	-44	34	43
63	104	-41	43	53
64	127	-63	61	73
68	129	-61	81	92
87	134	-47	100	118
108	139	-31	128	143
111	163	-52	154	172
118	153	-35	182	194
103	140	-37	208	226
106	139	-33	241	258
130	167	-37	264	282
115	143	-28	291	314
142	153	-11	322	340
147	178	-31	357	372

RABBIT 20 N= 14

MEAN I1= 98.6429 MEAN I2= 138 MEAN DIF=-39.3571
SD1= 35.1319 SD2= 28.4821 SD3= 13.6247
SE1= 9.38938 SE2= 7.61217 SE3= 3.64135

I1	I2	I3	D1	D2

TIME: 3.12 SECS.

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CURRICULUM VITAE

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August 9, 1944	Born in Hazel Green, Wisconsin, to Herbert and Elizabeth Fleege
1966	B. S. cum laude - Seattle University Seattle, Washington
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1970 - 1972	Captain in the United States Army Dental Corps - Fort Carson, Colorado
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Professional Societies

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ABSTRACT

AN EVALUATION OF TETRACYCLINE STAIN REMOVAL BY BLEACHING VITAL RABBIT INCISORS

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This study evaluated the effectiveness of bleaching tetracycline-stained teeth by measuring the loss of fluorescent intensity from teeth that were bleached.

Nineteen male New Zealand white rabbits, with 58 incisors stained with oxytetracycline and 16 incisors as unstained controls, were used. Three rabbits were sacrificed to determine whether the tetracycline stain was comparable between incisors in the same jaw. Of the remaining 16 animals, 6 were bleached once and 10 were bleached twice. One maxillary and one mandibular incisor were bleached in each jaw with 30 percent hydrogen peroxide and heat for ten minutes per tooth; the other incisors were protected with a rubber dam. The animals were sacrificed 24 hours after the last bleach. The fluorescent intensity of 374 select ground sections 100 ± 5 microns thick from the incisal, middle and gingival thirds of the teeth were measured with an ultraviolet light microscope coupled to a television electronic measurement system. These measurements were statistically analyzed by t-test, and observations correlated.

The dentin of tetracycline-stained maxillary incisors which were bleached twice and the dentin in the incisal one-third of the mandibular incisors which were bleached twice had a significantly ($P \leq 0.001$, $P \leq 0.005$) lower tetracycline fluorescent intensity than the dentin of unbleached tetracycline-stained teeth. The greatest loss of fluorescent intensity of tetracycline occurred in dentin closest to the dentino-enamel junction and varied from about 150 to 350 microns from the outer enamel surface. Clinical Kodachromes indicate that the loss of tetracycline pigment is associated with the loss of tetracycline fluorescence; The ground sections showed that the tetracycline fluorescence was never totally removed by two bleaches.